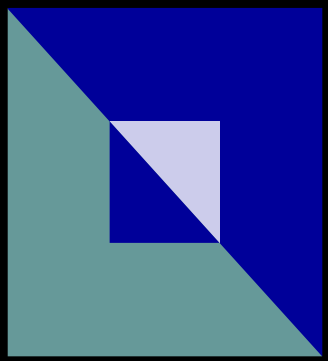




Rosita-2 project

Final Report

Editors:
Alain G.Verstraete
Elke Raes

Grant agreement n°SUB-B27020B-E3-S07.18222-2002

Final Report

March 2006

Ghent University
Department of Clinical Biology,
Microbiology and Immunology
De Pintelaan 185
9000 Gent
Belgium

E-mail: alain.verstraete@ugent.be

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Chapter 1

Executive summary.

- The Rosita-2 project was carried out in 2003-2005 in order to evaluate the usability and analytical reliability of the onsite oral fluid (saliva) drug testing devices.
- The study was carried out by National Institute for Criminalistics and Criminology in Brussels, Belgium, the National Public Health Institute in Helsinki, Finland, the Institute for Legal Medicine in Strasbourg, France, the Institute for Legal Medicine in Homburg/Saar, Germany, the Division of Forensic Toxicology and Drug Abuse, Norwegian Institute of Public Health, Oslo, Norway and Institute of Legal Medicine, University of Santiago de Compostela, Spain. It was coordinated by Ghent University, Ghent, Belgium.
- The study was performed in cooperation with the United States, where it is funded by The National Institute on Drug Abuse (NIDA), National Institutes of Health, US Department of Health and Human Services, the National Highway Traffic Safety Administration (NHTSA), US Department of Transportation and the Office of National Drug Control Policy Executive Office of the President. The US part is coordinated by The Walsh Group (Bethesda, Maryland). The study is carried out in the following states: Florida (Hillsborough County Sheriff's Office, Florida Department of Law Enforcement, Manatee County Sheriff's Office), Washington (Washington State Police, Washington State Toxicology Lab), Utah (Salt Lake City Police Department, Center for Human Toxicology) and Wisconsin (12 Police Jurisdictions, Wisconsin State Lab of Hygiene).
- In the US, the study continues until the end of 2006. The complete results for the European part and the partial results of the US parts are presented here.
- 2046 Subjects were included in the study and 2605 device evaluations were performed.
- Nine devices were evaluated: American Biomedica Oralstat, Branan Medical Oratect, Cozart Bioscience RapiScan (only in the USA), Dräger/Orasure DrugTest/Uplink, Lifepoint Impact, Securetec Drugwipe, Sun Biomedical Oraline, Ultimed Salivascreen and Varian OraLab.
- The devices had tests for the following drugs: amphetamines, methamphetamine, cannabis, cocaine and opiates. Three devices also had a test for benzodiazepines.
- During the study, two devices were withdrawn from the market: Dräger/Orasure DrugTest/Uplink and Lifepoint Impact.
- Subjects for whom a suspicion of driving under the influence of drugs existed were asked to participate in the study on a voluntary basis. In most cases the following samples were taken: a blood sample and an oral fluid sample with the Intercept™ sampler for analysis in the lab with reference techniques (gas or liquid chromatography coupled to mass spectrometry, sometimes after screening with an immunoassay), and one (or two) oral fluid sample for analysis with the onsite device.
- For some devices, a very high percentage of failures was observed. Depending on the type of device, this was apparently due to too little or too viscous saliva (the fluid didn't migrate until the control line, or it caused smears), or to a malfunctioning of the instrument that read the results. For six devices (Varian Oralab, Lifepoint Impact, Branan Oratect 2nd generation, Sun Oraline, Ultimed Salivascreen and Branan Oratect 1st generation), more than 25% of the devices failed to run. For the other devices, the

number of failures was less than 10 % (American Biomedica Oralstat and Dräger DrugTest/Orasure Uplink) or less than 5% (Cozart Rapiscan and Securetec Drugwipe). The evaluators considered that a failure rate of maximum 5-10% was acceptable.

- The number of evaluations per device varied widely, with two devices evaluated more than 500 times, one 190 times and 6 less than 50 times. The explanation lies in the large number of failures for Branam Medical Oratect, Ultimed Salivascreen and Varian OraLab, which led to their exclusion from the study and the late start of the evaluation of the American Biomedica Oralstat, Lifepoint Impact and Sun Biomedical Oraline.
- The percentages of positive samples were: amphetamines (including methamphetamine, ecstasy and analogues) 20 %, benzodiazepines 32 %, cannabinoids 36%, cocaine 19% and opiates 8%.
- The analytical evaluation of the **amphetamine and methamphetamine** tests (in comparison to the reference method in oral fluid) showed a sensitivity (percentage of the true positive samples that tested positive with the onsite assay) varying between 40% and 83% and a specificity (percentage of the negative samples that tested negative with the onsite assay) between 80% and 100%.
- The analytical evaluation of the **benzodiazepine** tests (in comparison to the reference method in oral fluid) showed a sensitivity varying between 33% and 69% and a specificity between 85% and 94%.
- The analytical evaluation of the **cannabis** tests (in comparison to the reference method in oral fluid) showed a sensitivity varying between 0% and 74% and a specificity between 70% and 100%. Detailed analysis of the data for cannabis showed that some devices (e.g. Drugwipe) gave a negative result even when very high concentrations of THC were found with the Intercept. The reason is unknown, but one hypothesis is that with an improved (more thorough) sampling technique more THC could be captured, resulting in more positive results.
- The analytical evaluation of the **cocaine** tests (in comparison to the reference method in oral fluid) showed a sensitivity varying between 0% and 97% and a specificity between 91% and 100%.
- The analytical evaluation of the **opiate** tests (in comparison to the reference method in oral fluid) showed a sensitivity varying between 51% and 100% and a specificity between 86% and 100%.
- No device met the criteria proposed during the Rosita-1 project (sensitivity and specificity > 90%, accuracy > 95%) for the amphetamines, benzodiazepines and cannabis. The Varian Oralab met these criteria for cocaine and opiates, but it gave 26% failures, so it cannot be recommended.
- The operational evaluation of the Drugwipe showed that the sampling technique was well accepted by the police and the subjects, but the results, particularly for cannabis, were difficult to read. There were also problems when using it in cold weather.
- The operational evaluation of the Dräger DrugTest/Orasure Uplink showed that sample collection was easy and hygienic, but that the procedure was long and complicated. The test must be read by an instrument, so it cannot be used in remote areas or when no instrument is available.
- The operational evaluation of the American Biomedica Oralstat showed that the collection stick lost one of its collection sponges in some cases. This test could also be read with or without the reading unit, but the scanning of the test strip by the electronic reader was sometimes difficult.
- The operational evaluation of the Branam Medical Oratect showed that the test was liked by the police officers, because it is very small and portable and no additional

equipment is needed, but the sample collection was too complicated, it could be outsmarted by the tested persons and it took too much time. The number of failures was too high.

- The operating procedure of the RapiScan was fairly direct, but was found to intimidate officers if they were not able to use it soon after training. Many officers were uncomfortable using the instrument, stating that it was difficult to remember the procedure.
- The operational evaluation of the Lifepoint Impact showed that in many cases the collected sample volume was not sufficient because the instrument stopped the sampling automatically after a preset time.
- The test procedure of the Sun Biomedical Oraline was simple with few steps but a rather large sample volume was needed and it took too much time. There were problems to use it in cold and rainy weather. The lines indicating positive or negative results were too pale.
- The operational evaluation of the Ultimed Salivascreen showed that the device gave more invalid than valid tests. Officers reported smearing of the result bands or not enough saliva collected by the device to give a reading.
- The operational evaluation of the Varian OraLab showed that subjects were often unable to provide sufficient oral fluid during specimen collection, resulting in many invalid tests. Officers also experienced difficulty observing the presence or absence of the test lines making interpretation of results inconsistent.
- At the end of the study, no device was considered to be reliable enough in order to be recommended for roadside screening of drivers. However, the experience in the state of Victoria in Australia shows that random roadside oral fluid testing of drivers for methamphetamine and cannabis (using the Securetec Drugwipe followed by the Cozart RapiScan and chromatographic analysis in the lab) has a deterrent effect. Government officials should carefully weigh the pros (deterrent effect) and the cons (risk that drivers will realise that they often test negative after having used drugs due to the limited sensitivity of the test) of introducing random drug testing with the currently available devices.

Chapter 2: Introduction

Alain Verstraete, Elke Raes

Department of Clinical Biology, Microbiology and Immunology.
Ghent University.

Drug use in Europe and in the world.

Drug use has increased very significantly in the world in the last decades. According to the 2005 world drug report (1), 200 million people, or 5% of the world's population in the 15-64 age group, have used drugs at least once in the last 12 months. This is 15 million people more than the year before. The number of cannabis users worldwide is now close to 160 million people (4% of the population aged 15-64). There are 26 million (meth)amphetamine users and 8 million ecstasy users. The number of opiate users is estimated to have risen slightly to around 16 million people (11 million of which abuse heroin) and close to 14 million people use cocaine.

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) estimates that around 20 million Europeans (over 6% of all adults) have used cannabis in the last year. Around 9.5 million (almost 4% of all adults), are classified as current users, and roughly 3 million young adults, mostly young males, are estimated to be daily, or almost daily, users. Around 9 million Europeans (3% of all adults) have ever used cocaine; between 3 and 3.5 million (1% of all adults) are likely to have tried the drug in the last year, while around 1.5 million (0.5% of all adults) are classified as current users, having used it in the last month (2). The weighted average rate of problem drug use (i.e. heroin) in the EU is probably between 0.4 and 0.7% of the population aged 15–64 years, which corresponds to 1.2–2.1 million problem drug users in the EU, of whom some 850 000 to 1.3 million are active injectors. These estimates are far from robust and will need to be refined as more data become available from the new Member States.

Studies have shown that approximately 80% of drug users will drive after having used drugs. Some even drive while using drugs. In the US in 2004, it was estimated that 10.6 million people have driven under the influence of drugs in the past year¹.

Traffic safety in Europe

Crashes killed 46,700 persons in the 25 countries of the EU in 2003, in 2004. There are nearly 2.0 million people injured in Europe. Traffic crashes are the main cause of death for people under 45; they cost € 200 billion/year to society, which corresponds to 2% of GDP. One out of 3 inhabitants of Europe will be hospitalised during his life because of a crash.

In order to reduce the toll on the road, the European Union has implemented the European Road Safety Action Programme with the aim of saving 25,000 lives on EU roads by 2010. A part of these crashes can be attributed to driving under the influence of drugs. While alcohol is certainly responsible for more deaths and injuries than drugs, in certain populations and at certain times (especially weekend nights) drugs do play a significant role in the number of accidents.

¹ Data from the 2004 National Survey on Drug Use and Health:

<http://www.drugabusestatistics.samhsa.gov/nsduh/2k4nsduh/2k4Results/2k4Results.htm#2.15>

Measures taken to combat DUID.

Since 1998, several European countries have, in analogy to alcohol, introduced *per se* legislation for DUID. This legislation has increased the number of prosecutions (3) but, even if the data are scarce, there is little indication that there has been an important change in behaviour and that this has reduced drugged driving (4). On the other hand there are some data that random roadside screening for drugs has a deterrent effect. Several studies have investigated the effectiveness of random roadside testing. Two studies of self-reported driving behaviour of drug users pointed out the deterrent effect of random roadside testing for drugs. Terry *et al.* (5) found that 65% of regular cannabis users reported that they would be deterred from driving after smoking cannabis if there was random roadside testing. The results of a questionnaire conducted by Jones *et al.* (6) revealed that random roadside testing appears to act as a more effective deterrent against drug-driving than either increasing the severity of sanctions or providing factual information about the risks associated with the behaviour. In Victoria (Australia) behaviour changes have already effectively been noticed as a result of the random roadside testing introduced in 2004. Surveys have showed that the level of awareness of random saliva testing increased from 78% to 92% of drivers, 33% of illicit drug users stated that the drug tests have influenced them, primarily to avoid taking drugs when they are going to drive and the proportion of drug-using respondents who drove while under the influence of drugs did drop in the after period (45% to 35%) (P. Swann, personal communication).

Roadside drug testing

Since many years, police officers involved in road safety have expressed the need for a rapid and reliable drug test. The onsite urine drug tests and the first generation of the oral fluid tests were evaluated in the first Rosita study (7;8).

The final conclusion of this project in the year 2000 were that for each type of drug, several urine drug tests satisfied the analytical criteria for a good test (accuracy > 95%, sensitivity > 90%, specificity > 90%, when compared with a reference method in urine), but none scored highly for all the drug categories. When the necessary facilities were available (e.g. a sanitary van), urine could be obtained relatively easily at the roadside. When the facilities were not available, obtaining a urine sample was a problem and it could be time-consuming if the driver had to be brought to a suitable facility. A clear majority of countries preferred oral fluid as the matrix for roadside testing, while one country favoured sweat and one favoured urine. The methods for obtaining saliva needed further improvements. Wiping over the tongue seemed to be a well accepted technique, but in this case the analytical detection technique needs to be very sensitive. Sampling oral fluid with dedicated devices gave many problems. The onsite tests for oral fluid that were available at that time were insufficiently sensitive and/or specific to give reliable results for most classes of drugs. There were several new versions of the evaluated tests and new onsite tests for oral fluid, some of which look very promising in terms of sensitivity, which should be evaluated when they become available.

The Rosita-2 consortium

At the very end of 2002, Directorate-General Transport and Energy of the European Commission gave a grant (Grant agreement n°SUB-B27020B-E3-S07.18222-2002) for a second Rosita-study.

The main aim of the Rosita-2 study was to evaluate the newer onsite devices for detecting drugs in oral fluid. The study was coordinated by Ghent University and performed in six countries by the following institutions:

- The National Institute for Criminalistics and Criminology in Brussels, Belgium

- The National Public Health Institute, KTL in Helsinki, Finland
- The Institute for Legal Medicine in Strasbourg, France
- The Institute for Legal Medicine in Homburg/Saar, Germany
- The Division of Forensic Toxicology and Drug Abuse, Norwegian Institute of Public Health, Oslo, Norway
- The Institute of Legal Medicine, University of Santiago de Compostela, Spain

In addition, Rosita-2 was also performed in the United States, where it was coordinated by The Walsh Group. The US part was funded by:

- The National Institute on Drug Abuse [NIDA], National Institutes of Health, US Department of Health and Human Services
- The National Highway Traffic Safety Administration [NHTSA], US Department of Transportation
- The Office of National Drug Control Policy, Executive Office of the President

The participating centres in the US are:

State	Police Department(s)	Coordinator/Lab
Florida	Hillsborough County Sheriff's Office	The Walsh Group/FDLE
	Manatee County Sheriff's Office*	The Walsh Group/FDLE
Washington	Washington State Police	Washington State Toxicology Lab
Utah	Salt Lake City PD	Center for Human Toxicology
Wisconsin	12 Police Jurisdictions	Wisconsin State Lab of Hygiene
Indiana	Indiana State Police*	State Department of Toxicology

* Dropped from participation in project

Aim of the project

The aim of the project was to evaluate the available onsite devices for the detection of drugs in oral fluid. Subjects were asked to participate in the study and provided informed consent. An oral fluid sample was taken for analysis by an onsite device and an additional oral fluid sample was taken for the confirmation analysis with the Intercept device and a blood sample was taken.

The onsite test was performed by a police officer and the results were recorded.

The results of the onsite device are compared to the results of the reference methods (GC-MS or LC-MS(MS)), using cut-offs that were agreed upon at the onset of the study.

In the United States, the project continues one more year, and it is scheduled to be completed at the end of 2006.

This book contains the results of the Rosita-2 study. Chapter 3 gives an overview of the existing onsite devices. Chapters 4-8 give the results of each individual country and state. The results for France are not given in an individual chapter as the results for cannabis have been published (9). The results of the other drugs are incorporated in the global analysis. Chapter 9-12 gives the partial results from the United States. Chapter 13 gives the results of the global analysis of the data from the European and US centres.

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Chapter 3: Survey of the existing devices for the on-site detection of drugs in oral fluid

Elke Raes, Alain G. Verstraete

Department of Clinical Biology, Microbiology and Immunology, Ghent University.

Abstract

A total of fifteen different devices, from 13 manufacturers have been available during the duration of the Rosita project (2003-2005). Two of them were withdrawn from the market (Lifepoint Impact and Dräger/Orasure Uplink/DrugTest). The most relevant characteristics of these devices are summarised in this chapter: manufacturer, matrix, drugs that can be detected, number of assays/device, cost, CE marking and FDA approval, storage conditions, shelf life, manipulations to obtain result, presence of internal control lines, possibility to store the result, result interpretation, whether specimen is available for confirmation, and a picture of the device.

Introduction

During the course of the Rosita project, the teams were constantly looking for new devices that became available on the world market. The manufactures were contacted and the devices were first evaluated in the lab of the Centre for Human Toxicology in Salt Lake City. Three evaluations were performed and two of these studies were published {Crouch, 2005 10 /id; Walsh, 2003 41 /id}. The devices were then allocated to the different teams.

Methods

A first draft of the characteristics of each device was written based on the information available in brochures, product inserts and the websites of the different manufacturers. These data were than submitted to a representative of the manufacturer in order to complete or correct them. The devices are discussed in alphabetical order of the device (not manufacturer) name.

Alphabetical list of the devices.

1. Drugwipe



Figure 1: Securetec Drugwipe

Manufacturer: Securetec Detektions-Systeme AG
 Matrix: Saliva or sweat or traces on surfaces
 Available for: METH, AMP, COC, OPI, CAN, BZO
 Number of assays/device: 1, 2 or 5
 Cost: For Drugwipe 5: between € 16.5 and 18 (depending on the ordered quantity)
 FDA: Not approved
 CE: Not marked
 Storage conditions: Room temperature
 Shelf life: 24 months
 Manipulations to obtain result: 1) disconnect wiping section from the device
 2) wipe the surface of the tongue or the body for appr. 10 s
 3) reassemble the device and dip the absorbent pad into water for 15 counts
 4) read the results after approx. 10 minutes
 Positive/negative control line: Present
 Possibility to store the result: Yes
 Interpretation of the result: Direct visual (second red line, next to control line, line appears in the case of a positive specimen!)
 Save specimen for confirmation: Possible

Table 1: Cut-offs in ng/ml saliva of the different Drugwipe tests

Analyte	DRUGWIPE II	Drugwipe II twin	Drugwipe 5
free morphine	20	20	20
free codeine	20	20	20
cocaine	50	50	50
benzoylecgonine	75	75	75
Δ^9 -THC	30	30	30
11-Nor- Δ^9 -THC-COOH	2	2	2
d-Amphetamine	200	200	200
d-Methamphetamine	100	100	100
MDMA	100	100	100
MDEA	250	250	250

Contact: Securetec Detektions-Systeme AG
 StartPoint Technologie Park
 Eugen-Sänger-Ring 1
 85649 Brunnthal
 Tel: +49 (0)89 203080-1651
 Fax: +49 (0)89 203080-1652
 E-Mail: info@securetec.net
 Web: www.securetec.net

2. Impact



Figure 2: Lifepoint Impact. Left: Instrument; right: cassette

Manufacturer:	Lifepoint Inc.
Matrix:	Saliva
Available for:	THC, COC, PCP, (Meth)AMP, OPI (+alcohol)
Number of parameters/device:	5
Cost:	/
FDA:	Not approved
Storage conditions:	/
Shelf life:	/
Manipulations to obtain result:	1) insert the cassette portion of the saliva test module into the Impact test system 2) push start 3) place aspirator (a suction pump) into the donor's mouth. The sampling procedure takes about 5 minutes. 4) test results appear on screen within 5 minutes
Positive/negative control line:	/
Possibility to store the result:	Yes
Interpretation of the result:	Digital read-out
Save specimen for confirmation:	Not possible

Table 2: Cut-off in ng/mL of Impact

Analyte	Cut-off (ng/ml)
THC	15
COC	20
PCP	10
(Meth)AMP	50
OPI	40

Contact: The company went bankrupt in April 2005

3. Multi-Line Twist Screen Test Device



Figure 3: Multi-Line Twist Screen Test Device

Manufacturer:	Acon Laboratories Inc.
Matrix:	Saliva
Available for:	COC, THC, (Meth)AMP, OPI, PCP
Number of assays/device:	6
Cost:	Not communicated
FDA:	Not approved
CE:	Not communicated
Storage conditions:	2-30°C
Shelf life:	24 months
Manipulations to obtain result:	1) insert the sponge into the mouth to collect oral fluid (during 3 minutes) 2) place test device on flat surface, insert the collector into the test device by pushing it into the collection chamber and turning the collector clockwise until engaged. WAIT ONE MINUTE. 3) after one minute, rotate the collection chamber counter clockwise and set the timer for 9 minutes. 4) read results at 9 minutes.
Positive/negative control line:	Negative control line should be present
Possibility to store the result:	No
Interpretation of the result:	Visual
Save specimen for confirmation:	Possible

Table 3: Cut-offs in ng/mL of the Multi-Line Twist Screen Test Device

Analyte	Cut-off (ng/ml)
THC	100
THCCOOH	12
COC	20
(Meth)AMP	50
PCP	10
OPI	40

Contact:	Acon Laboratories, Inc. 4108 Sorrento Valley Boulevard San Diego, CA 92121, USA
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Tel: 1-858-535-2030
Fax: 1-858-535-2035
E-Mail: info@aconlabs.com
Web: www.aconlabs.com

This device is also available as **Oral fluid Test with Twist Cassette** for £9 from:

SureScreen Diagnostics Ltd.
1 Prime Parkway
Prime Enterprise Park
Derby DE1 3QB
Tel: +44 (0)1332 365318
Fax: +44 (0) 1332 292230
E-Mail: sales@surescreen.com
Web: www.surescreen.com

4. OraLab



Figure 4: Varian OraLab

Manufacturer:	Varian Inc
Matrix:	Saliva
Available for:	COC, Morphine, (Meth)AMP, THC, PCP
Number of assays/device:	6
Cost:	€ 20.8
FDA:	Not approved
CE:	Not marked
Storage conditions:	Room temperature
Shelf life:	12 months
Manipulations to obtain result:	1) collect the saliva sample with the collector foam (during 3 minutes)

2) place the cassette on a flat surface and place the collector foam into the sample well on top of the device. Push the collector slowly (approx. 5 seconds) and firmly downward until it comes to a stop. The saliva flows directly into the test cassette

3) test validity and results must be interpreted between 10 and 15 minutes after initiating the test

Positive/negative control line: Present

Possibility to store the result: No

Interpretation of the result: Visual

Save specimen for confirmation: Possible: confirmation well holds sample overflow

Table 4: Cut-offs in ng/mL of OraLab

Analyte	Cut-off (ng/ml)
THC	100
COC	20
(Meth)AMP	50
PCP	10
Morphine	40

Contact: Varian Inc
 Technical Support
 25200 Commercentre Drive
 Lake Forest, CA 92630-8810
 Tel: 1-800-737-9667
 E-Mail: dat.techsupport@varianinc.com
 Web: www.varianinc.com
www.varian-onsite.com

5. OraLine (4 insert)



Figure 5: Sun Biomedical Oraline

Manufacturer: Sun Biomedical Laboratories

Matrix: Saliva

Available for: THC, COC, Methamphetamine (METH) and OPI

Number of assays/device: 4

Cost: \$6 to \$10 USD

FDA: Not approved

CE: Not communicated

Storage conditions: Room temperature

Shelf life: 12 to 18 months

Manipulations to obtain result: 1) Collect sufficient saliva sample in the test device
 2) Wait for one minute to make sure test is running ok
 3) Read result after 5 to 10 minutes

Positive/negative control line: Present

Possibility to store the result: No
 Interpretation of the result: Visual
 Save specimen for confirmation: Possible in separated vial

Table 5: Cut-offs in ng/mL of OraLine (4 insert)

Analyte	Cut-off (ng/ml)
THC	4
THCA	4
COC	25
BE	10
METH	50
OPI	40

*: Cutoffs level determined from the real oral fluid samples, correlated with the GC/MS data

Contact: Sun Biomedical Laboratories
 604 VPR Center
 1001 Lower Landing Road
 Blackwood, NJ 08012
 Tel: 856-401-1080
 Fax: 856-401-1090
 E-Mail: admin@sunbiomed.com
 Web: www.sunbiomed.com

6. ORALscreen DRUGOMETER



Figure 6: Oralscreen Drugometer

Manufacturer: Avitar Technologies Inc.
 Matrix: Oral fluid
 Available for: THC (delta 9, vs. THCCOOH), COC (cocaine, benzoylecgonine), OPI (morphine, 6-AM, codeine, dihydrocodeine, hydrocodone, hydromorphone, oxycodone, oxymorphone), MethAMP (Methamphetamine, MDA, MDEA, MDMA (Ecstasy))
 Number of assays/device: 4
 Cost: \$24.96 (low quantity)
 FDA: Not approved
 CE: Not marked
 Storage conditions: 2-30 °C, 36-86 °F, bring to room temperature before use.
 Shelf life: 18 months

Manipulations to obtain result:	1) move handle forward, exposing the foam, place foam end in mouth and move it around gently for at least 3 minutes 2) holding the device upright with the foam end slightly tipped toward you, slowly pull the handle downward until the red indicator arrow is fully visible. The red stop line, located directly above the tip of the arrow, will be visible and the handle will lock 3) immediately place Drugometer on a flat horizontal surface, keeping the test window facing upward 4) read the results. A negative interpretation may be made once a red line is clearly visible (usually in less than one minute). A non-negative interpretation must be made after 15 minutes (not beyond!)
Positive/negative control line:	Present
Possibility to store the result:	Yes, by means of ORALscreen OSR
Interpretation of the result:	Visual
Save specimen for confirmation:	Separate ORALconfirm kit for sample collection, inclusive of chain of custody forms, seals, etc.

Table 6: Cut-offs in ng/mL of ORALscreen Drugometer

Analyte	Cut-off (ng/ml)
THC	250-500
COC	10-15
OPI	
morphine	2.5
6-AM	10
(dihydro)codeine	2.5
oxycodone, Oxymorphone	60
hydrocodone, hydromorphone	2.5
Methamphetamine	
Methamphetamine	50
MDMA/Ecstasy	50

7. ORALscreen 4



Figure 7: Avitar Oralscreen 4

Manufacturer:	Avitar Technologies Inc.
Matrix:	Oral fluid
Available for:	THC (delta 9, vs. THCCOOH), COC (cocaine, benzoylecgonine), OPI (morphine, 6-am, codeine, dihydrocodeine, hydrocodone, hydromorphone, oxycodone, oxymorphone), MethAMP (Methamphetamine, MDA, MDEA, MDMA (Ecstasy))
Number of assays/device:	4
Cost:	\$19.95 in low quantity
FDA:	Not approved
CE:	Not marked
Storage conditions:	2-30°C (bring to room temperature before use)
Shelf life:	18 months
Manipulations to obtain result:	1) collect an “oral fluid” sample (hydrophilic, FDA approved): slide the plastic hood back and place the foam end in the mouth; move around for at least 2 minutes to allow the oral fluid to enter the foam 2) remove and slide the hood forward to cover the foam and squeeze the between the fingers to expel 4 drops in the sample well (wait 10 seconds between each individual drop) 3) read the results. A negative interpretation may be made once a red line is clearly visible, usually in a minute or two. A non-negative interpretation must be made 15 minutes (not beyond!) after the first drop of oral fluid has been introduced to the test device
Positive/negative control line:	Present
Possibility to store the result:	Yes, by means of ORALscreen OSR
Interpretation of the result:	Visual
Save specimen for confirmation:	Separate ORALconfirm kit for sample collection, inclusive of chain of custody forms, seals, etc.

Table 7: Cut-offs in ng/mL of ORALscreen 4

Analyte	Cut-off (ng/ml)
THC	250-500
COC	10-15
OPI	
morphine	2.5
6-AM	10
(dihydro)codeine	2.5
oxycodone, oxymorphone	60
hydrocodone, hydromorphone	2.5
Methamphetamine	
Methamphetamine	50
MDMA/Ecstasy	50

Contact:	Avitar Technologies Inc. 65 Dan Road Canton
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MA 02021 USA
Tel: (800) 255-0511
Fax: (781) 821-2440
E-Mail: sales@avitarinc.com
Web: www.avitarinc.com

8. Oral Fluid Drug Screen Device



Figure 8: AconLabs Oral Fluid Drug Screen device

Manufacturer:	Acon Laboratories Inc.
Matrix:	Saliva
Available for:	COC, THC, (Meth)AMP, OPI, PCP
Number of assays/device:	6
Cost:	Not communicated
FDA:	Not approved
CE:	Not communicated
Storage conditions:	2-30°C
Shelf life:	24 months
Manipulations to obtain result:	1) insert the sponge into the mouth to collect oral fluid (during 3 minutes) 2) flip open the top of the collection vial and insert the collector, sponge first. Push the collector down against the strainer to extract the oral fluid 3) close the top of the collection vial and unscrew the cap exposing the dropper. Gently squeeze the end chamber of the collection vial and dispense 3 drops into the sample well of cassette (place test device on a clean and level surface). Replace the vial cap afterwards 4) read results at 10 minutes. Do not read results after 1 hour.
Positive/negative control line:	Present
Possibility to store the result:	No
Interpretation of the result:	Visual
Save specimen for confirmation:	Possible

Table 8: Cut-offs in ng/mL of Oral Fluid Drug Screen Device

Analyte	Cut-off (ng/ml)
THC	12
COC	20
(Meth)AMP	50
PCP	10
OPI	40

Contact:

Acon Laboratories, Inc.
4108 Sorrento Valley Boulevard
San Diego, CA 92121, USA
Tel: 1-858-535-2030
Fax: 1-858-535-2035
E-Mail: info@aconlabs.com
Web: www.aconlabs.com

This device is also available as **Oral fluid with Collection vial** for £9 from:

SureScreen Diagnostics Ltd.
1 Prime Parkway
Prime Enterprise Park
Derby DE1 3QB
Tel: +44 (0)1332 365318
Fax: +44 (0) 1332 292230
E-Mail: sales@surescreen.com
Web: www.surescreen.com

According to Surescreen, this device is CE and FDA approved.

And this device is also available from Ultimed as **SalivaScreen III** (\$ 6.50 , 3assays, list price for export) or **SalivaScreen VI** (6 assays \$ 9.50, list price for export). This device is CE marked.

Contact:

ulti med Products GmbH
Postfach 1814
D-22908 Ahrensburg, Germany
Tel: 04102-800 90
Fax: 04102-500 82
E-Mail: info@ultimed.org
Web: www.ultimed.org
www.salivascreen.com

9. **OralStat**

Manufacturer:

American Bio Medica Corporation

Matrix:

saliva

Available for:

(meth)AMP, COC, OPI, CAN, BZO, PCP, MTD

Number of parameters/device:

6

Cost:

Not communicated

FDA:

Not approved

CE:

Not communicated

Storage conditions:

Room temperature

Shelf life:

Not communicated



Figure 9: OralStat Device

Manipulations to obtain result:

- 1) remove label and verify test slide is in the up position
- 2) insert the handle of the collection swab into each of the “A” wells, press down completely and agitate side to side at least 3 seconds
- 3) hold sponge between cheek and gum for at least one minute each side of mouth. Continue until the sponge is fully expanded
- 4) insert sponge into well “B”, push down until it clicks into place and wait at least 5 seconds
- 5) agitate side to side at least 3 seconds, wait 8 minutes, squeeze tabs in and push test slide down
- 6) read the results after 2 to 4 minutes

Positive/negative control line:

Present

Possibility to store the result:

No

Interpretation of the result:

Visual

Save specimen for confirmation:

Possible

Table 9: Cut-offs of OralStat in ng/mL

Analyte	Cut-off (ng/ml)
(meth)AMP	25
BZO	25
COC	12
MTD	25
OPI	20
PCP	5
THC	25

Contact:

American Bio Medica Corporation
Corporate Offices
122 Smith Road
Kinderhook, New York 12106

Tel: 800-227-1243/518-758-8158
Fax: 518-758-8171
E-Mail: info@americanbiomedica.com
Web: www.americanbiomedica.com

10. Oratect II



Figure 10: Branan Oratect

Manufacturer:	Branan Medical Corporation
Matrix:	Saliva
Available for:	Meth, AMP, THC, COC, OPI, PCP (BZO coming soon)
Number of parameters/device:	6
Cost:	Depends on volume
FDA:	For Forensic Use Only
CE:	Not marked (approval is in process)
Storage conditions:	Room temperature
Shelf life:	12 Months
Manipulations to obtain result:	1) Remove the blue cap by holding the sides and pulling gently. 2) Open mouth and gently rub the collection pad inside mouth against cheek in a circular motion several (approximately 15-20) times. Make sure to keep head level. 3) Gently rub the collection pad against the opposite cheek in a circular motion several (approximately 15-20) times. 4) Gently rub the collection pad on top of the tongue several (approximately 15-20) times. Do not chew, suck, bite or bend the collection pad. 5) Rub the collection pad underneath the tongue several (approximately 15-20) times. 6) Place the collection pad underneath the tongue for approximately 30 seconds to collect saliva. Instruct the donor to hold the device in place with their hand. Sufficient amount of saliva collected is indicated by the flow the blue lines. 7) Remove from mouth as soon as blue lines flow at both of the test windows. Recap device. 8) Lay the device on a flat surface and read the results in 5 minutes after removing the device from the mouth. Do not read results after 7 minutes.
Positive/negative control line:	Present
Possibility to store the result:	Results may be photocopied
Interpretation of the result:	Visual

Save specimen for confirmation: Yes. Buffer solution vials for confirmation purposes are provided.

Table 10: Cut-offs in ng/mL of Oratect II

Analyte	Cut-off (ng/ml)
THC	40
COC	20
OPI	10
PCP	4
Meth	25
AMP	25

Contact:

Branan Medical Corporation
10015 Muirlands Road, Suite C
Irving, CA 92618, USA
Tel: 1-866-intect7 or (949) 598-7166
Fax: (949) 598-7167
E-Mail: info@brananmedical.com
Web: <http://brananmedical.com>

11. RapiScan



Figure 11: Cozart RapiScan

Manufacturer:

Cozart Bioscience Ltd.

Matrix:

saliva

FDA:

Yes, only for the 2-Panel Drug Test for opiates and methadone

CE:

Marked

Storage conditions:

Room temperature

Manipulations to obtain result:

- 1) collect a saliva sample with the Cozart RapiScan Saliva Collection pack – based on a collector sampler (dilution with buffer)
- 2) pipette a required volume of oral fluid into the cartridge (disposable pipettes)
- 3) the cartridge, which houses the immunoassays, is placed into the instrument and an incubation period is activated

4) digital read-out for each drug tested and saving of results

Positive/negative control line: Present

Possibility to store result: Yes

Interpretation of the result: Digital read-out

Save specimen for confirmation: Possible

Table 11: Properties of the different Rapiscan tests

	5-Panel Drug Test		2-Panel Drug Test		Single Panel Drug Test
Tests for	OPI, COC, AMP, BZO, CAN	OPI, COC, AMP, BZO, MTD	OPI, MTD	OPI, COC	Methamphetamine
Incubation period (minutes)	12	12	6	3	5
Shelf life (months)	18	9	24	24	12
Cost (€)	17.4		11.8		8.8
Cut-offs (ng/mL) D amphetamine	45	45			
(+)-methamphetamine					45
(-)-methamphetamine					1500
MDMA/MBDB					45
MDEA					1500
MDA	150	30			
Temazepam	15	15			
Cocaine	30	30		30	
THC	150				
Methadone		15	30		
Codeine			30	30	
Heroin	60	60	60	60	
Morphine	30	30	36	36	
Benzoylcegonine	30	30		30	

*: the cut-offs are in neat saliva

Contact:

Cozart plc
 92 Milton Park
 Abingdon
 Oxfordshire
 UK
 OX14 4RY
 Tel: +44 (0)1235 861483
 Fax: +44 (0) 1235 835607
 E-Mail: info@cozart.biz
 Web: www.cozart.co.uk

12. Sali•Chek™ System



Figure 12: Sali Chek system

Manufacturer:	Bamburgh Marrsh LLC
Matrix:	Saliva / oral fluids
Available for:	Methamphetamines (METH), AMP, COC, THC, OPI, PCP
Number of assays/device:	6
Cost:	To be determined based upon volume requirements, the End-User Price will be targeted at approx. \$20 USD
FDA:	Not cleared for marketing in the United States at this time
CE:	Not marked
Storage conditions:	Ambient Temperature
Shelf life:	24 months
Manipulations to obtain result:	1) Saliva is collected by means of a “fork-shaped” pad, which simultaneously delivers oral fluid to two test strips located in the “handle” of the device (complete in under 2 minutes) 2) Results can be read within 15 minutes
Positive/negative control line:	Present
Possibility to store the result:	Yes (using the Sali•Chek™ Hand-Held Reading Device)
Interpretation of the result:	Visual
Save specimen for confirmation:	Possible

Table 12: Cut-offs in ng/mL of SaliChek

Analyte	Cut-off (ng/ml)
THC	12
COC	20
OPI	40
PCP	10
AMP	50
METH	50

Contact:

Paul D Slowey PhD
Managing Member
Bamburgh Marrsh LLC
15720 NE 31st Avenue
Vancouver, WA 98686 USA
Tel: 360.546.1563
Fax: 360.546.1581
E-Mail: pds@4saliva.com
Web: www.bamburghmarrsh.com
www.4saliva.com

13. SalivaScreen 5



Figure 13: SalivaScreen 5 device

Manufacturer:	ulti med Products GmbH
Matrix:	Saliva / oral fluids, sweat
Available for:	Methamphetamines (METH), COC, THC, OPI, BZO
Number of assays/device:	5
FDA:	Not approved
CE:	Marked
Storage conditions:	Room temperature
Shelf life:	/
Manipulations to obtain result:	1) collect the saliva sample with the collection swab 2) press the collection swab from top to bottom to expel 3-4 drops of collected saliva. Allow 2 minutes for dissolving of reagents and 10 minutes for the completion of the chromatography 3) test validity and results must be interpreted between 10 and 20 minutes after initiating the test
Positive/negative control line:	present

Possibility to store the result: yes (using the SureScreen Reader)
 Interpretation of the result: visual
 Save specimen for confirmation: /

Table 13: Cut-offs in ng/mL of SalivaScreen 5

Analyte	Cut-off (ng/ml)
THC	2
COC	30
Morphine	30
Methadone	30
METH	50

Contact: **ulti med Products GmbH**
 Postfach 1814
 D-22908 Ahrensburg, Germany
 Tel: 04102-800 90
 Fax: 04102-500 82
 E-Mail: info@ultimed.org
 Web: www.ultimed.org
www.salivascreen.com

14. Smartclip Multidrug



Figure 14: Smartclip Multidrug

Manufacturer: EnviteC-Wismar GmbH
 Matrix: Saliva, sweat, surfaces
 Available for: 4 drug groups: Amphetamine, Cocaine (also as crack); Morphine, Codeine, Heroin; Ecstasy, Methamphetamine, MBDB
 Number of assays/device: 4
 Cost: € 19
 FDA: Not approved
 CE: Marked
 Storage conditions: Room temperature
 Shelf life: 24 months
 Manipulations to obtain result: 1) remove protective cap from sponge holder
 2) collect a saliva sample with the sponge by putting it under the tongue with mouth closed for approx. 1 minute (the sponge needs to have swollen roughly 3 times its original thickness and shine with moisture, add 10- 12 drops of the buffer onto the sponge
 3) remove the protective clip from the devise and close

	the device
	4) immediately hold the test in horizontal position. It is best to lay it on a flat surface, so that the viewing window is pointing upwards
	5) read results after 10 minutes
Positive/negative control line:	Present
Possibility to store the result:	No
Interpretation of the result:	Visual
Save specimen for confirmation:	It is possible to send the closed device to a lab and they can extract the remaining specimen out of the sponge

Table 14: Cut-offs in ng/mL of Smartclip Multidrug

Analyte	Cut-off (ng/ml)*
COC	20
Morphine	40
MethAMP	100
AMP	50

*: the cut-offs are in saliva diluted with buffer

Contact:	EnviteC-Wismar GmbH Umweltschutz und Medizintechnik Alter Holzhafen 18 23966 Wismar, Germany Tel: +49 3841 360 1 Fax: +49 3841 360 222 E-Mail: info@envitec.com Web: www.envitec.com
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15. Uplink/DrugTest

Manufacturer:	manufactured by OraSure Technologies Inc./USA exclusively for Dräger Safety AG & Co. KG aA/D
Matrix:	Oral Fluid
Available for:	AMP, Methamphetamine, THC, COC, OPI,
Number of parameters/device:	5
Cost:	Test Kits List price €20 Euro / Test Kit – 4 Euro / Parameter class
FDA:	Not approved
EU IVD:	Analyzer, Test Kits and Control Test Kits meet the provisions of Annex III of Directive 98/79/EC (In vitro diagnostic medical device)
Storage conditions:	Test Kits → between 2°C to 30°C
Shelf life:	Test Kits → 6 month from manufacturing day
Manipulations to obtain result:	1) collect an oral fluid sample with the swab collection sponge 2) insert the buffer cartridge into the lower groove of the test cassette by aligning lower tabs on the cartridge with notches on the cassette. Rock cap and remove 3) insert the sample collection device into the buffer cartridge and press down on the sample collection device

(notice that the sample collection device clicks slightly into place and a cracking sound can be heard when it is pushed down firmly)



Figure 15: Dräger DrugTest, Orasure Uplink

- 4) remove the handle of the sample collection device by means of a quarter turn anticlockwise
- 5) close buffer cartridge and allow 4 minutes of incubation time
- 6) turn the buffer cartridge clockwise until the upper groove is positioned above the groove of the test cassette. Push down the buffer cartridge firmly until the cartridge wings touch the edge of the cassette. Allow the fluid to develop for 8 minutes
- 7) enter sample ID in the analyzer and insert the cassette into the analyzer
- 8) the analyzer will indicate that the door is closed and the read cycle has begun. The analyzer will report in 4 minutes

Positive/negative control line:	Present (as internal Test adequacy meter = intrinsic QC)
Possibility to store the result:	Yes
Interpretation of the result:	Digital read-out
Save specimen for confirmation:	Possible

Table 15: Cut-offs in ng/mL of Uplink

Analyte	Analytical Cut-off (ng/ml)
THC	20
COC	5
OPI	5
METH	10
AMP	10

NOTE: Ninety-five percent (95%) confidence level for a “positive result” will be attained with drug at 250% of this cutoff concentration for all assays except opiates. At 250%, the confidence limit for the opiates assay is 90%

Question: are cut-offs in pure saliva or after dilution?

The analytical cutoff is based on pure oral fluid. Values were calculated back from average dilution data.

Regarding Testing “Accuracy”, please see page 6 of user instructions

Contact: Dräger Safety AG & Co. KGaA
Revalstrasse 1
23560 Lübeck, Germany
Tel: +49 451 882 2981
Fax: +49 451 882 4002
Web: www.draeger-safety.com

OraSure Technologies, Inc.
220 East First Street
Betlehem, PA 18015, USA
Tel: 800-869-3538
Fax: 610-882-3572
Web: www.orasure.com

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- Slowey Paul D. (Bamburgh Marrsh LLC)
- Sun Ming (Sun Biomedical Laboratories)
- Wischerhoff Erik (ulti med Products GmbH)

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Chapter 4: Center Report Belgium

Michel Willekens

Local Police, Zone Kempen Noord-Oost , Arendonk

Marleen Laloup, Maria del Mar Ramirez Fernandez, Gert De Boeck and Nele Samyn

Federal Public Service Justice, National Institute of Criminalistics and Criminology (NICC), Brussels

Abstract

The aim of this study was to evaluate the accuracy of two on-site oral fluid testing devices on the market (Dräger DrugTest[®] and Drugwipe[®] 5). The on-site test results were compared with plasma (GC-MS) and oral fluid results (LC-MS-MS). A total of 178 Dräger DrugTests and 140 Drugwipes were performed. Unfortunately, no complete satisfactory results were obtained for either of both on-site devices for amphetamines and cocaine (sensitivity < 86%, accuracy < 93%). Also in the case of cannabis positive samples, no acceptable results were obtained (sensitivity < 51%, accuracy < 60%). Due to the limited number of opiate-positive samples, no statement can be made about this group of illicit drugs.

In addition, comparison was performed between the oral fluid results and the analysed plasma samples and/or on-site urine tests. The analytical evaluation indicates a fairly good correlation of preserved oral fluid and plasma samples.

Introduction

In March 1999, the Belgian parliament adopted a law on driving under the influence of certain illicit drugs. This new law punishes driving under the influence of cannabis, cocaine, opiates and amphetamines and includes not only specific substances, but mentions also analytical legal limits for these substances in plasma. In Belgium, an initial suspicion of impairment is established using a field sobriety test, based on external signs of substance abuse and on some well-defined psychomotor tests. If there is suspicion of impairment, a urine test (cannabis, cocaine, opiates, amphetamines, methamphetamines) is performed. A positive test result for at least one parameter leads to immediate driving suspension (for 12 hours) and blood sampling by an independent physician.

One of the key elements in the enforcement process is the possibility to take immediate administrative measures (short-term driving ban) and the selection of drivers that will have to undergo venipuncture. An important step to improve the detection of impaired drivers has been the extensive training program for police officers in some countries. A second complementary approach is the use of an acceptable screening device at the roadside to provide the police officer with additional evidence of recent drug use [1]. Therefore, the application of easy-to-use roadside tests has gained increasing interest in Belgium.

Oral fluid testing has been proposed as an alternative to urinalysis [2], and has already proven its application in roadside studies [3-7]. It shows some practical advantages such as its ready availability, low invasiveness and good correlation with impairment. Indeed, it is assumed that drug detection in oral fluid is based on drug diffusion from blood and/or contamination of the oral cavity with drug substance, reflecting an actual drug influence, while urine provides a wider window of detection

and is not correlated with blood levels. However, the results of the first Rosita study have shown that the available on-site oral fluid screenings, particularly for testing of THC, needed to be improved significantly [8].

Recently, two new devices, the Drugwipe[®] 5 and the Dräger DrugTest[®] were introduced by respectively Securetec (Brunnthal, Germany) and Dräger (Lübeck, Germany). The first being a non-instrumental immunodiagnostic assay and the latter a device-based system using a specific analyzer for signal detection.

In the present study, both devices were evaluated and correlated with analytical data of plasma and oral fluid obtained under roadside conditions.

Methods

Subject selection

Figure 1 explains the legal procedure applied in a driving under the influence of drugs (DUID) case and the study protocol for the evaluation of oral fluid. From February 2004 until January 2006, subjects were selected at police controls all over the country, but with one main control site, focused to intercept drivers coming from the Netherlands. Urine, blood and oral fluid (on a voluntary basis) were collected to analyze for illicit drugs. The selection procedure is a three-step-process:

1) In general, only for drivers that screened negative for alcohol (legal limit 0.5 g/L), a standardized test battery (checklist) was performed to check the drivers' level of impairment. On drivers that screened positive for alcohol, only the procedure for alcohol was applied. However, in some county court districts, both alcohol and drug procedures were applied when impairment was suspected.

The first part of the test battery mentions all physical signs of influence, the second part consists of four psychomotor tests: the Romberg, the one leg stand, the walk and turn, and the finger to nose. In order to decide whether a person might be impaired, at least one parameter in each part of the test battery has to be clearly positive. The central bureau of investigations of the State Police trained police officers to obtain sufficient experience in the interpretation of results of the drug recognition test battery.

2) If signs of impairment are detected, the Officer proceeds to the second step of the procedure, the urine test. If there are no clear signs of "being under the influence", the procedure stops at this point. There is a toilet available on the spot, and if not, the person has to be taken to the Police Station for collecting an urine sample. The law clearly states that all measures have to be taken to respect the privacy of the person. On the other hand, the Court Circular for the Police proceedings also indicates that the Police Officer has to take all measures necessary to avoid adulteration of the urine sample.

3) If this urine test is positive for at least one drug class, a physician is summoned to take a blood sample. At least 15 ml of blood will be taken and sent to a certified laboratory as soon as possible. The cut-off values for the plasma analysis are also mentioned in the law: THC (tetrahydrocannabinol) 2 ng/mL, free morphine 20 ng/mL, amphetamine, MDMA (ecstasy), MDEA, MBDB, benzoylecgonine or cocaine 50 ng/mL.

Samples were also obtained from a few volunteers, mostly passengers in the car, admitting recent drug use. They agreed to participate through informed consent.

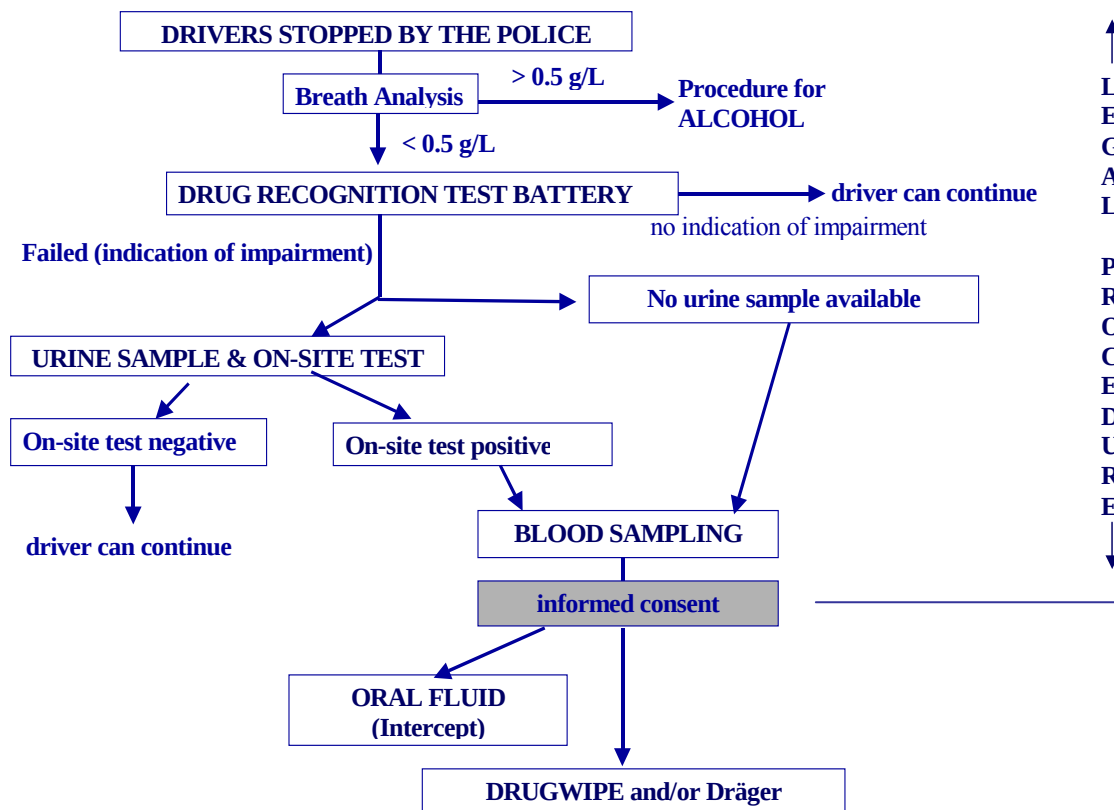


Figure 1. Study protocol based on the legislation for driving under the influence of drugs in Belgium.

On-site screening

To screen the urine, a rapid immunoassay test is applied on-site. The on-site urine test Dipro drug screen 5 panel (VanDePutte group, Boechout, Belgium) provides results for five products: cannabinoids (cut-off 50 ng/mL), morphine (cut-off 300 ng/mL), cocaine (cut-off 300 ng/mL), methamphetamines (cut-off 500 ng/mL), and amphetamines (cut-off 1000 ng/mL). The panel test was extensively evaluated in the laboratory [9] and showed an excellent cross-reactivity for MDMA and MDA. The urine on-site test result is noted and available.

The Drugwipe[®] 5 is wiped on the tongue and/or the inside of the cheek for oral fluid testing. The recommendations of the manufacturer were quite inconsistent concerning the sampling procedure. In contrast to the earlier versions of the Drugwipe, panel tests for the simultaneous detection of cocaine, opiates, THC and amphetamines/methamphetamines are now available and the read-out signal is not presented as a color but as a line, with a control line to mark the validity of the test. After 3 to 10 minutes, the appearance of a pink line in the test window for one or more compounds indicates illicit drug use. The cut-off levels for oral fluid testing with the Drugwipe II[™] were: 30 ng/mL for THC, 50 ng/mL for cocaine, 100 ng/mL for MDMA and methamphetamine, 500 ng/mL for MDEA, 200 ng/mL for amphetamine and 20 ng/mL for morphine and codeine. However, no cut-offs for the Drugwipe[®] 5 were reported. According to the manufacturer, new versions of the Drugwipe[®] 5 were used throughout the study.

The Dräger DrugTest[®] system combines the test strip method of immunological drug detection with an innovative signal technology known as UPT (Up-Converting Phosphor Technology). It consists of two main components: the Dräger DrugTest[®]

Kit for Oral Fluids (comprising the collection device and the test cassette with inserted buffer cartridge) and the Dräger DrugTest[®] Analyzer (a portable analyzer for reading the test cassette and for data management). Different panel cassettes are available for detecting up to six substance classes in a single cassette. The test subject collects an oral fluid sample by gently moving the collection device in the mouth for about a minute. The sampling sponge swells in size as it soaks up the oral fluid, signaling to the tester that the sampling process is complete. The collection device is first inserted into the test cassette and pushed down to release some of the oral fluid into the buffer cartridge. The handle of the collection device is removed and the buffer cartridge is sealed. After a four-minute reaction time, the buffer cartridge is turned and lowered, triggering the immunological detection reaction within the test cassette. After eight minutes, the test cassette can be inserted into the Dräger DrugTest[®] Analyzer, which displays the results of the analysis, distinct for each class of drugs, in the form of a qualitative reading (positive or negative). The proposed cut-off values are 5 ng/mL for cocaine and opiates, 10 ng/mL for amphetamines and methamphetamines and 20 ng/mL for the cannabinoids.

Sampling

Blood samples were collected in 5 mL glass Vacutainer tubes using sodium fluoride and potassium oxalate as anticoagulant. The tubes were immediately cooled to +4°C (cool box) and centrifuged the next day. The corresponding plasma was frozen at –20°C until analysis.

Oral fluid was collected with the Intercept[®] (OraSure Technologies, Bethlehem, PA, USA) according to the manufacturer's instructions. The device collects an average of 0.38 ± 0.19 (SD) mL (range 0.05 to 0.8 mL) of oral fluid and a dilution factor of 1 in 3 is arbitrarily accepted [10]. The tubes were centrifuged and the preserved oral fluid was stored at –20°C prior to analysis.

Confirmation procedures and cut-off values

The on-site data for urine were not confirmed in the laboratory. Only the parameters that tested positive in urine during the roadside screening were confirmed in plasma using GC-MS. If the urine test result was not available, blood analysis was performed for all parameters. In addition, all plasma samples were screened for alcohol (with FPIA, confirmation with headspace-GC-FID) and medication (with HPLC-DAD).

In contrast, all preserved oral fluid samples, collected with the Intercept[®] device, were analyzed by LC-MS-MS for basic drugs and THC, using two separate methods (100 µL for THC, 250 µL for basic drugs). If a discrepancy was noted between the urine test result and the oral fluid confirmation result, or if no oral fluid was available, further analysis of the corresponding blood samples was performed for additional drugs.

1. Analysis of plasma samples

Illicit drugs

Confirmation of plasma samples was performed using previously published extraction and derivatization techniques for cocaine and its metabolites, opiates and cannabinoids [11-13]. Amphetamine, methamphetamine, ephedrine and the designer amphetamines were extracted from plasma using solid phase extraction (SPE) with mixed-mode C8-cation exchange columns (Bond Elut Certify, Varian, Belgium) and ethylacetate/ammonia (98:2, v/v) as eluent; heptafluorobutyric anhydride was used as derivatization agent. Derivatization was performed with BSTFA + 1% TMCS.

Quantitative analyses were performed using the deuterated analogues of all the analytes of interest on a Agilent 6890 gas chromatograph equipped with an autosampler (HP7673A) and interfaced with an Agilent 5973 mass selective detector (Agilent Technologies, Diegem, Belgium). Analytical conditions were optimised for the detection of (1) cocaine, benzoylecgonine, ecgonine methyl ester, morphine and codeine; (2) amphetamine, methamphetamine, ephedrine, MDA, MDMA, MDEA, MBDB; (3) THC, OH-THC and THC-COOH. The MS was operated in SIM mode. At least three ions were monitored for the analytes and two ions for the internal standards. The methods are permanently evaluated through participation in external quality control programs (SFTA, France; GTFCh, Germany). In this study, plasma was considered positive for amphetamines, cannabinoids, cocaine and opiates following the cut-offs mentioned in Table 1.

Table 1. Cut-off values in plasma and oral fluid (n.a.: not analysed).

Substance	LOQ blood (ng/mL)	LOQ oral fluid (ng/mL)
Amphetamine	20	25
Methamphetamine	20	25
MDMA	20	25
MDA	20	25
MDEA	20	n.a.
Cocaine	20	8
Benzoylecgonine	20	8
Morphine	10	20
Codeine	10	20
6-MAM	n.a.	5
THC	1	2
THC-COOH	5	n.a.
11-OH-THC	1	n.a.

Alcohol

All plasma samples were screened for the presence of alcohol using FPIA (AxSYM, Abbott, Louvain-La-Neuve, Belgium). Confirmation and quantification of positive samples was performed using an Agilent 6890N GC-FID, equipped with an Agilent G1888 headspace sampler (Agilent Technologies). 1-propanol was used as internal standard. The method is permanently evaluated through participation in external quality control programs (SFTA, France; GTFCh, Germany).

Medication

Medication was extracted from plasma (1 mL) using liquid-liquid extraction with 1-chlorobutane, after addition of the internal standard (clobazam) and 500 μ L of a saturated ammonium chloride buffer (pH 9.2). Analyses were performed using a Hewlett-Packard (Agilent) HPLC device HP 1100 Series (Agilent Technologies). Analytes were separated on a Lichrospher RP8ec column (250 x 4.0 mm, 5 μ m) (Merck, Darmstadt, Germany), eluted isocratically with 100 mM phosphate buffer pH 2.3-acetonitrile (63:37, v/v), delivered at a flow rate of 1 mL/min with a run time of 30 min. Systematic toxicological analysis was performed using an UV Spectra Library [14, 15]. Wavelength calibration and accuracy checks of the DAD were performed regularly.

2. Analysis of preserved oral fluid samples

The analysis of preserved oral fluid samples was performed according to previously published validated procedures [16, 17]. For THC, quantitative analyses were performed using a Waters Alliance 2690 separation module (Waters, Milford, MA, USA), interfaced with a Quattro Premier tandem mass spectrometer (Waters). Analytes were separated on a XTerra MS C₁₈ column (150 x 2.1 mm, 3.5 µm) (Waters), eluted isocratically with 1 mM ammonium formate-methanol (10:90, v/v), delivered at a flow rate of 0.2 mL/min. Ionization was achieved using electrospray in positive ionization mode (ESI+). The optimum conditions were: capillary voltage, 2.0 kV; source block temperature, 120°C; desolvation gas (nitrogen) heated to 280°C and delivered at a flow rate of 700 L/h. Collision-induced dissociation (CID) of each protonated molecule was performed. The collision gas (argon) pressure was maintained at 0.35 Pa (3.5 x 10⁻³ mBar) and the collision energy (eV) adjusted to optimise the signal for the most abundant product ions, which were subsequently used for multiple reaction monitoring (MRM) analysis.

A Quattro Ultima tandem mass spectrometer (Waters) fitted with a Z-Spray ion interface was used for the detection of amphetamine, methamphetamine, MDMA, MDA, cocaine, benzoylecgonine, morphine, codeine and 6-MAM in preserved oral fluid. Ionisation was achieved using electrospray in the positive ionisation mode (ES+). The following conditions were found to be optimal for the analysis: capillary voltage, 1.0 kV; source block temperature, 120°C; desolvation gas (nitrogen) heated to 350°C and delivered at a flow rate of 800 L/hour. The cone voltage (CV) was adjusted to maximise the intensity of the protonated molecular species [M+H]⁺ and collision induced dissociation of each protonated molecule was performed. Collision gas (argon) pressure was maintained at 2.7x10⁻³ mBar and the collision energy (eV) adjusted to optimise the signal for the most abundant product ions, which were subsequently used for MRM analysis.

The data are expressed as nanograms per millilitre of diluted specimen. The exact volume of specimen collected by the individual devices was not determined.

Criteria

A sample was considered as being positive by GC-MS (plasma) or LC-MS-MS (preserved oral fluid), if following compounds were present:

- for cannabinoids: THC
- for amphetamines: amphetamine, methamphetamine, MDMA, MDA and/or MDEA (the latter one was only determined in plasma)
- for cocaine: cocaine and/or benzoylecgonine
- opiates: 6-MAM (only determined in preserved oral fluid), morphine and/or codeine

Results

Case selection

Of the subjects that were stopped during the police controls, 270 agreed to perform an on-site oral fluid screening test (Dräger DrugTest[®] and/or Drugwipe[®] 5). Of these, 4 subjects refused to give a blood sample, 13 failed to provide a urine sample and of 28 no preserved oral fluid sample (Intercept[®]) was available. Concerning the use of the oral fluid test devices, the Dräger DrugTest[®] was used 178 times, the Drugwipe[®] 5 140 times and the Oralab[®] device 5 times. It was decided to exclude the latter device from the study due to its important number of invalid tests (4 out of 5 cases). In contrast, the Dräger DrugTest[®] gave in nearly 4% of the cases an invalid test result (7

out of 178 cases), which was even lower for the Drugwipe® 5 (0.7%, 1 out of 140 cases).

All on-site oral fluid tests were performed by Michel Willekens or in the presence of one of the co-workers of the National Institute of Criminalistics and Criminology (NICC). Both on-site screening devices had some practical disadvantages. The procedure of the Dräger DrugTest® was considered to be time-consuming (15-20 minutes), which was not the case for the Drugwipe® 5. However, the latter gave some read-out problems, resulting in a difficult interpretation. These results were considered to be positive, but were marked as ‘doubtful’ in the corresponding tables.

Presence of alcohol and medication

Of all plasma samples tested, 8.1% had a BAC (blood alcohol concentration) level of > 0.5 g/L (median: 1.24 g/L). Medication was present in 5.3% of all plasma samples. In most of these cases a benzodiazepine was present: nordiazepam (in combination or not with diazepam) was the most common detected compound (1.9%), followed by bromazepam (1.8%). Clobazam (and its metabolite norclobazam)*, desalkylflurazepam and clonazepam, other members of this family, were each detected in only one case. Other compounds included venlafaxine (2 cases), tramadol (1 case) and bisoprolol (1 case).

Oral fluid on-site screening devices

1. On-site oral fluid test results versus preserved oral fluid and plasma results for cannabinoids

Both on-site devices perform equally well for the screening of cannabinoids in oral fluid, when compared to both oral fluid and plasma confirmation results (Table 2). The number of false positive (FP) results is limited, resulting in a high specificity. However, this is in contrast with the number of false negatives (FN), which is dramatically high for both devices. This leads to a poor sensitivity and a poor accuracy for both devices compared to both oral fluid and plasma results. There is no indication that the FN results correspond to the oral fluid samples with the lowest concentrations of THC. The application of higher confirmation cut-off values will therefore not alter the results significantly. On the other hand, the high number of FN results could be due to a state of dryness in the oral cavity, leading to insufficient sample volume in the sampling sponge.

It should be remarked that, for this parameter, a high number of ‘doubtful’ results was noted for the Drugwipe® 5 device, which complicates the interpretation and leads to an even more dubious analytical evaluation. In conclusion, the versions of the Dräger DrugTest® and the Drugwipe® 5 that were tested are not reliable for the detection of THC in oral fluid.

* Despite its use as an internal standard in the HPLC method, the presence of clobazam was detected through an elevated chromatographic peak for this compound and the presence of its metabolite, norclobazam. In addition, confirmation of this compound was performed using a validated LC-MS-MS method [18].

Table 2. Cannabinoids: on-site oral fluid tests versus preserved oral fluid LC-MS-MS and plasma GC-MS.

	Oral fluid		Plasma	
	Dräger DrugTest	Drugwipe	Dräger DrugTest	Drugwipe
Total	(n = 144)	(n = 129)	(n = 164)	(n = 127)
TP	58	43 ^(a)	60	38 ^(c)
TN	19	23	36	33
FP	1	1 ^(b)	7	8 ^(d)
FN	66	62	61	48
Sensitivity	47%	41%	50%	44%
Specificity	95%	96%	84%	80%
PPV	98%	98%	90%	83%
NPV	22%	27%	37%	41%
Prevalence	86%	81%	80%	80%
Accuracy	53%	51%	59%	56%

(a) including 18 ‘doubtful’ results; (b) including 1 ‘doubtful’ result; (c) including 21 ‘doubtful’ results; (d) including 2 ‘doubtful’ results

2. On-site oral fluid test results versus preserved oral fluid results for amphetamines

The comparison of the on-site amphetamine tests with the reference method in preserved oral fluid is given in Table 3. No comparison was made with plasma results, since not all plasma samples were analysed with GC-MS (see ‘Methods’). For the Dräger DrugTest[®], where both a test for amphetamine and methamphetamine exists, we considered the combination of both results, i.e. the test is negative if both results are negative and the test is positive if either result is positive. For the Drugwipe[®] 5, only one combinatory test amphetamine/methamphetamine was available.

For the amphetamines, the comparison between both devices is somewhat hampered by the important difference in the number of positive samples (19 for the Dräger DrugTest[®] versus 35 for the Drugwipe[®] 5, as confirmed by LC-MS-MS). However, it can be stated that there is no big difference between the two devices.

The specificity for both tests is satisfactory, but the sensitivity is much lower. The accuracy is reasonably good.

Table 3. Amphetamines: on-site oral fluid tests versus preserved oral fluid LC-MS-MS.

	Dräger DrugTest	Drugwipe
Total	(n = 136)	(n = 128)
TP	11	23 ^(a)
TN	110	92
FP	7	1
FN	8	12
Sensitivity	58%	66%
Specificity	94%	99%
PPV	61%	96%
NPV	93%	88%
Prevalence	14%	27%
Accuracy	89%	90%

(a) including 1 ‘doubtful’ result

3. On-site oral fluid test results versus preserved oral fluid results for cocaine

The obtained results are similar for both devices and are comparable with those obtained for amphetamines: high specificity, but low sensitivity in combination with an acceptable accuracy (Table 4).

Table 4. Cocaine: on-site oral fluid tests versus preserved oral fluid LC-MS-MS.

	Dräger DrugTest	Drugwipe
Total	(n = 136)	(n = 128)
TP	17	22 ^(a)
TN	108	95
FP	4	7 ^(b)
FN	7	4
Sensitivity	71%	85%
Specificity	96%	93%
PPV	81%	76%
NPV	94%	96%
Prevalence	18%	20%
Accuracy	92%	91%

(a) including 1 ‘doubtful’ result; (b) including 1 ‘doubtful’ result

4. On-site oral fluid test results versus preserved oral fluid results for opiates

Only 2 positive results in plasma and/or oral fluid were noted, and are described in Table 5. Beside these results, 3 false positive results were noted for the Dräger DrugTest[®] and 8 false positives for the Drugwipe[®] 5 (including 3 ‘doubtful’ results). Of these, respectively 1 and 3 urines screened positive for opiates, suggesting the presence of other opiates.

Table 5. Opiates: on-site oral fluid tests versus preserved oral fluid LC-MS-MS and plasma GC-MS.

Dräger DrugTest	Plasma (ng/mL)	Preserved oral fluid (ng/mL)
+	Morphine: 87 Codeine: 28	6-MAM : 238 Morphine : 725 Codeine : 63
+	Morphine : 168 Codeine : 40	6-MAM : 291 Morphine : 1063 Codeine : 132

Comparison of plasma GC-MS versus preserved oral fluid LC-MS-MS

Box-plots, representing all confirmed oral fluid and plasma samples are shown in figures 2-4. No results are shown for MDA, since this compound was detected in only 11 preserved oral fluid samples (median: 74.8 ng/mL, range: 33.0-185.1 ng/mL) and in 6 analyzed plasma samples (median: 29.2 ng/mL, range: 22.0-83.0 ng/mL). For THC-COOH, which is considered not to be present in oral fluid [2], plasma concentrations ranged from 5 to 470 ng/mL (median: 41 ng/mL). In all cases, except for benzoylecgonine, higher median concentrations were noted in preserved oral fluid in comparison with plasma.

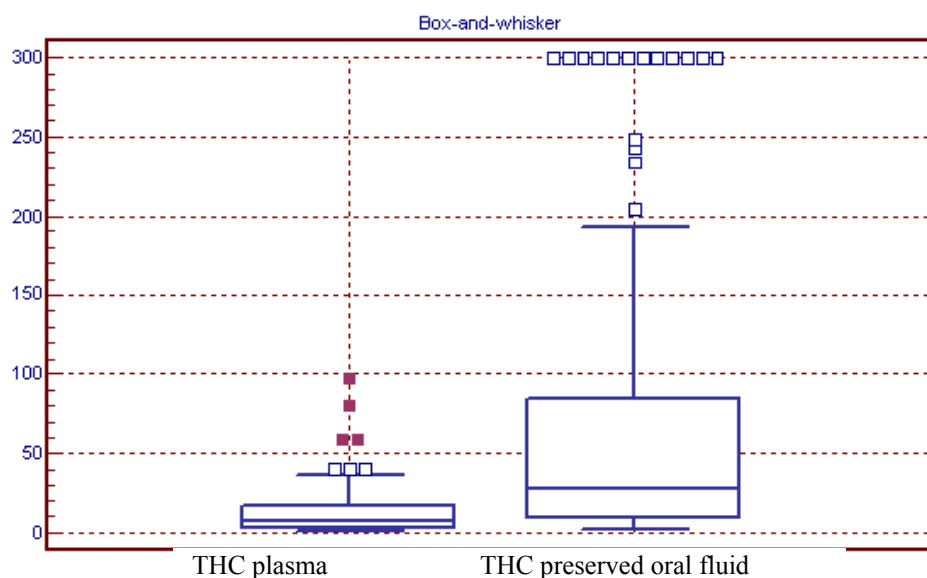


Figure 2. Box-and whisker plots of THC levels in plasma and preserved oral fluid samples. Concentrations plotted on the Y-axis are expressed as ng/mL. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum value, excluding "outside" (square marker) and "far out" values (filled square marker) which are displayed as separate points.

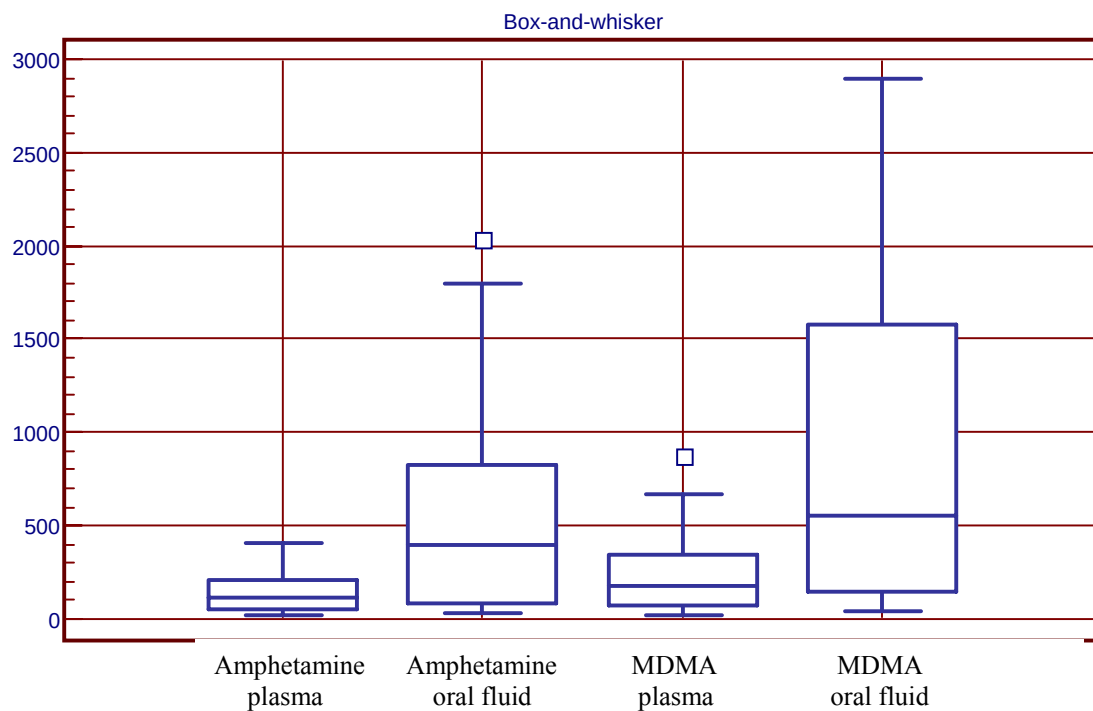


Figure 3. Box-and whisker plots of amphetamine and MDMA levels in plasma and preserved oral fluid samples.

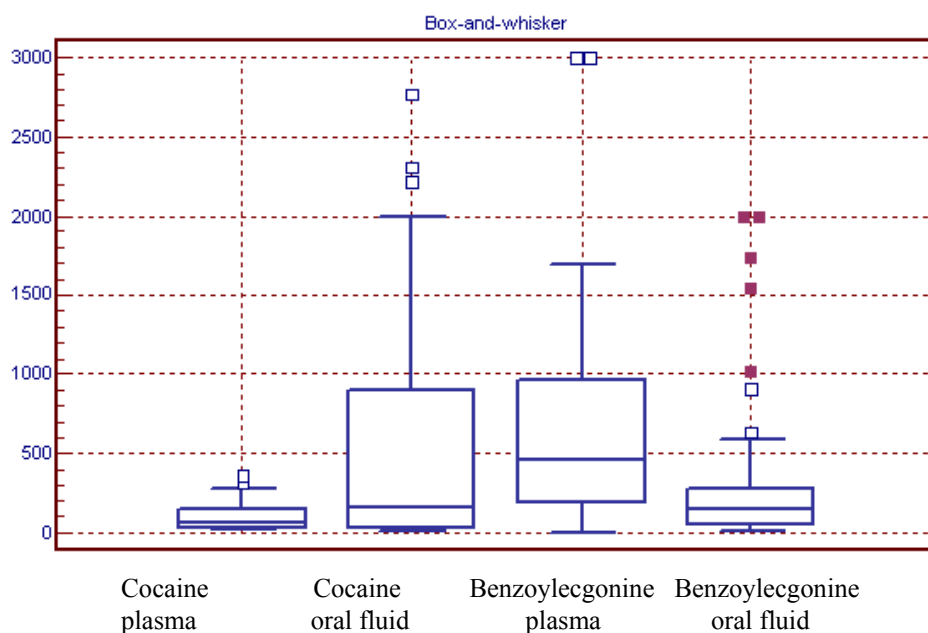


Figure 4. Box-and whisker plots of cocaine and benzoylecgonine levels in plasma and preserved oral fluid samples.

Finally, the analyzed preserved oral fluid samples were compared to the analyzed plasma samples and/or on-site urine tests. The sensitivity and accuracy were > 90% for the three groups of illicit drugs. A somewhat lower specificity of 80% was noted for cannabis. These results indicate a good correlation of preserved oral fluid and plasma samples (Table 6).

Table 6. Preserved oral fluid compared to plasma result and/or urine test.

	Cannabis (n = 239)	Amphetamines (n = 231)	Cocaine (n = 231)
Total			
TP	181	49	38
TN	36	180	189
FP	9	2	4
FN	13	0	0
Sensitivity	93%	100%	100%
Specificity	80%	99%	98%
PPV	95%	96%	90%
NPV	73%	100%	100%
Prevalence	81%	21%	16%
Accuracy	91%	99%	98%

Discussion

Undoubtedly, oral fluid offers a non-invasive way of screening at the roadside with the possibility of direct supervision. This is a major advantage in comparison to urine testing. Police officers recognize these benefits and are very willing to participate in the development of a suitable on-site test. Indeed, the use of oral fluid would facilitate the legal procedure.

Oral fluid testing is considered as the only alternative to blood sampling to obtain some information about impairment due to recent use of medicines or drugs of abuse. This was confirmed through the comparison of results obtained in oral fluid and plasma/urine, which indicate a fairly good correlation of preserved oral fluid and plasma samples.

Sampling is time-consuming because of the high viscosity of the sample and the decrease of the salivary flow after amphetamine use or cannabis smoking. For roadside purposes, the screening test should only require a small amount of sample to facilitate the collection. The principle of the Drugwipe® (wiping the tongue) is highly recommended. However, complex sampling devices such as the Dräger DrugTest® are more difficult to accept.

One important issue is the accuracy of the on-site tests to detect drugs of abuse in oral fluid samples. Unfortunately, no complete satisfactory results were obtained for either of both on-site devices for amphetamines and cocaine (sensitivity < 86%, accuracy < 93%). Also in the case of cannabis positive samples, no acceptable results were obtained (sensitivity < 51%, accuracy < 60%). Due to the limited number of opiate-positive samples, no statement can be made about this group of illicit drugs.

In conclusion, sampling should be quick and easy and the development of a reliable screening test remains the biggest challenge the forthcoming years.

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Chapter 5: ROSITA 2 in Finland

Teemu Gunnar¹, Anna Rantanen¹, Charlotta Engblom¹, Heikki Seppä², Pirjo Lillsunde¹

¹ National Public Health Institute, KTL

² Ministry of Interior / Police Supreme Command

Abstract

This report contains the results from the Finnish part of the ROSITA 2 project and evaluation of Drugwipe 5 and Drugwipe Benzodiazepines (Securetec) on-site oral fluid screening tests. Drugwipe 5 includes the initial screening ability of the most common illicit drugs, i.e. amphetamines, cocaine, cannabis and opiates (no buprenorphine), whereas the other Drugwipe was a single panel test for widely abused licit prescription drugs, benzodiazepines.

The approach adopted in the project had a few principal aims: 1) to test the reliability of oral fluid screening tests, 2) to test the practicality of oral fluid screening from the operational point of view, 3) to determine the correlations of different drug concentrations in different biological matrices (especially whole blood vs. oral fluid), 4) to determine the suitability of oral fluid when considering the acute (ab)use of different drugs, 5) to develop more reliable laboratory procedures for identification, screening and quantification of drugs of abuse, 6) to improve the expertise and know-how of the operating police officers through training sessions, as well as preparation and distribution of training material and 7) to chart the 'best practices' and experiences of handling the drugs & driving cases in an international environment and adapt them for national purposes.

The results of on-site testing devices (Drugwipe) in comparison to the laboratory confirmation of oral fluid and whole blood samples performed by GC-MS were as follows: for oral fluid (number of samples; sensitivity; specificity; accuracy; prevalence) amphetamines (n=148; 95.5%; 92.9%; 95.3%; 90.5%), cannabis (n=148; 52.2%; 91.2%; 85.1%; 15.5%), cocaine (n=148; 50.0%; 99.3%; 98.6%; 1.4%), opiates (n=148; 100%; 95.8%; 95.9%; 2.7%), benzodiazepines (n=77; 74.4%; 84.2%; 79.2%; 50.6%) and for whole blood accordingly, amphetamines (n=266; 97.7%; 86.7%; 95.9%; 83.1%), cannabis (n=265; 68.3%; 87.9%; 84.9%; 15.5%), cocaine (n=266; 50.0%; 98.5%; 97.7%; 1.5%), opiates (n=266; 87.5%; 96.9%; 96.6%; 3.0%) and benzodiazepines (n=121; 66.7%; 87.0%; 74.4%; 62.0%). Comparable results from oral fluid and whole blood indicate that oral fluid is a suitable matrix for roadside drug testing and can be used as an indicator of recent drug use. Furthermore and in spite of the low sensitivity of the cannabis test, Drugwipe is a suitable preliminary test for helping police officers on the road to detect drugged driving cases in Finland, because of good usability and small size in addition to sensitive amphetamine and acceptable benzodiazepine test at high concentrations. Amphetamine and benzodiazepines are the most commonly found drugs in road traffic in Finland.

One of the most important outcomes of the Rosita 2 project in Finland was that several proposals for new orders, regulations and instructions were made.

Introduction

In advance of ROSITA 2 oral fluid sample collection phase and evaluation of the roadside screening tests, a few important political and legislative decisions were made in Finland: 1) In February 2003, zero-tolerance law was introduced for handling the drugs & driving cases. The law was applied for controlled drugs or their (active) metabolites that are listed in the UN conventions on narcotics and psychotropic substances (Narcotic act 1289/93; Penal code 50), 2) it was enacted in a regulation from the Ministry of Interior that on-site oral fluid screening tests for drugs were considered equal to on-site breath alcohol tests, and were thus compulsory, if asked by a police officer and 3) other orders and regulations from the Ministry of Interior, in addition to “*Breath and saliva testing*”, were modified, such as “*Supervision of drug use in road traffic*”, “*Drug dependence and driving permission*” and new “*field sobriety observation sheet*” for police officers. All these changes were mainly results of the ROSITA projects.

The ROSITA 2 project and the starting of roadside drug testing were introduced in a press conference at 19.4.2004, which was noted in all major TV channels and newspapers in Finland. The sample collection phase was started at 1.5.2004 and ended at 30.6.2005. Prior to on-site oral fluid testing, over 100 police officers were trained and a variety of training materials was delivered, including the book prepared by ROSITA 2 members concerning drugs & driving [1], which is also used in the Police Academy, and specific instructions for handling the on-site testing devices [2]. In Finland, the project was carried out by research teams with both practical expertise and academic knowledge. The core collaborators of the National Public Health Institute (KTL) in Finland were: The Ministry of Interior/Police Supreme Command (Mr. Heikki Seppä, General Inspector, Mr. Pertti Luntiala, ret. General Inspector), Helsinki Police Department (Mr. Arto Hokkanen, Sergeant), The Ministry of Transport and Communications (Ms. Eija Maunu) and the Department of Forensic Institute/University of Helsinki (Prof. Antti Penttilä, ret. Head of Department).

Methods

Existing legislation on drugs and driving cases in Finland

In February 2003, zero tolerance law (‘per se law’) was introduced in Finland. The law was applied to scheduled drugs, which include the drugs that are listed in UN convention on narcotics and psychotropic substances (Narcotic act 1289/93; Penal code 50). The zero tolerance is applied if the controlled drugs and/or their (active) metabolites are found in blood. The zero tolerance law is not applied if a driver has a right to use the controlled substance, e.g., by prescription of a physician. Nevertheless, the impairment law stays still in the background in the legislation. The driver will be convicted for driving while intoxicated if the driving ability is impaired by the use of drugs. Driving ability is not allowed be impaired by any substance. A driver can be convicted for the intake of drugs (also of medicinal drugs) if he/she is intoxicated to the extent that he/she may be dangerous to traffic safety (Penal code 23).

The impairment has to be shown in court. Symptoms of drug use are being documented by police officer (field sobriety observation sheet) and clinical sobriety

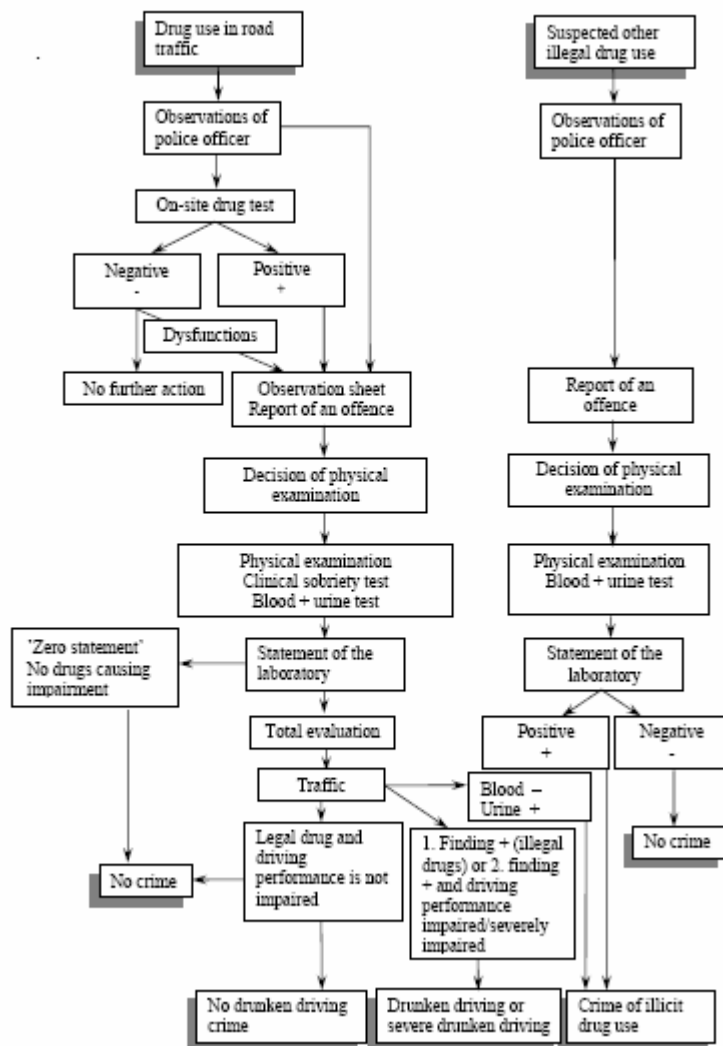


Figure 1. Handling of drugs and driving cases in Finland

test by physician. Drugs that have potentially harmful effects on driving ability have warning labels on the packages (Circular of the National Board of Health 1758/81). Furthermore, the impairment has to be shown also when the driver is prosecuted for severe drunken driving because of illicit drugs.

Before implementing the zero tolerance law, police had difficulties to show the impairment of driving ability in the court. A reasonable part of the drugs and driving cases may previously have remained unnoticed in Finland. This was the main reason for the government proposal for changing the legislation concerning the drugs in road traffic.

The statutory limits for drunken driving and severe drunken driving in Finland are 0.50 ‰ (w/w) and 1.2 ‰ (w/w), respectively. The corresponding breath alcohol control limits are 0.22 mg/l and 0.44 mg/l (Law on amending no. 23 of the penal code 655/1994).

Handling of drugs and driving cases in Finland

Police. In order to identify drunken drivers in Finland, the police are authorized by law to submit drivers to the preliminary test (breath test or oral fluid on site drug test) even

without any suspicion. In the Finnish regulations, the on-site testing devices for alcohol and drugs have the same position. The reasons for performing screening tests are usually random control (mainly for alcohol), impaired or dangerous driving (the most common for drugs), road traffic accidents or information of a bystander.

The police officer, who arrests the driver, offers also evidence of impairment. To demonstrate the impairment caused by drugs, the police officer has a standardized field sobriety test observation sheet available (Appendix 1). Any external symptoms of drug use are documented. Overview of handling drugs and driving cases in Finland is illustrated in Figure 1.

Physician & Health Care Unit. A clinical test for drunkenness is performed by a physician at the request of a police officer. When the screening test is positive or when drug-induced impairment of skills related to driving are suspected, the samples are taken as evidence that the drugs were present in the body fluids at the time of driving. If necessary, a blood sample can be taken even against the will of the driver (*Law on coercive means*). The physician documents his/her observations in a documentation sheet of clinical performance test (Appendix 2).

Laboratory. Alcohol and drug determinations of suspected drivers driving under the influence of alcohol and/or drugs are centered at the National Public Health Institute (KTL) in Finland. Drug analysis is performed at the request of the police. Drug screening in blood is performed and the drug concentrations in blood are measured. Depending on the substance and the concentration of the substance the zero tolerance or impairment law is applied. The written laboratory report to the police includes the results of toxicological analysis. When 'zero-tolerance' drugs are detected, only the test report of toxicological analysis is needed. In impairment legislation, pharmacological evaluation and conclusion with regard to possible impairment are made. Evaluation is done individually, taking into account the general characteristics of the drug, the concentrations of the drug in the blood, whether the drug use was acute or chronic, and the purpose of use, whenever these can be objectively estimated, e.g., using the ratio of the parent drug to the metabolite concentration.

Prosecutor. In practice, a driver driving under the influence of drugs is liable to prosecution if the presence of a 'zero-tolerance drug' in the driver's blood can be measured or if a significant amount of prescribed drug(s) / other substances can be measured, if impairment of performance has been demonstrated, and if the causative role of drugs is regarded as possible.

Court. For the illicit drugs and controlled medicinal substances, zero tolerance law is applied. For other substances, the impairment of the driver has to be shown in court (impairment law). This is based on 1) the documentation of external signs of drug use by police officer, 2) clinical sobriety test by physician and 3) the laboratory report including pharmacological evaluation.

Implementation of ROSITA 2 testing period

Location. The police forces in the three biggest cities in Finland, i.e. Helsinki (560 000 inhabitants), Espoo (225 000 inhabitants) and Tampere (200 000 inhabitants), were trained and organised for the ROSITA 2 study. These cities allowed a collection of the highest number of positive cases, training of police officers, which many had previous experience on on-site drug testing, and good possibilities for organising the police officers and on-site testing period. In addition, the mobile police in metropolitan area was participated. In Figure 2, the geographical location of the cities is illustrated.



Figure 2. The cities included in ROSITA 2 project in Finland (Espoo is the western neighbouring city of Helsinki)

Training sessions. Over 100 hundred police officers were trained to handle drugs and driving cases, to identify external symptoms of drug use and to operate with on-site testing devices in all three cities included. At the training sessions, both research scientists and experts from police gave lectures. Furthermore, the representative from the manufacturer of the on-site testing devices (Drugwipe, Securetec) introduced the devices and gave some practical tips from the manufacturer's point of view.

Organisation. The police forces were controlled at a national level from the Ministry of Interior by the members of ROSITA 2 Finnish team. In the police districts, they organised themselves for ROSITA 2 sample collection and on-site testing according to instructions delivered by the police supreme command and research scientists. The role of research scientists, therefore, remained supportive and our expertise was only used

with police officers in a case of necessity during the testing period. In other words, the research scientists were in a field only at the beginning of sample collection and police took care of all sample collection and testing the on-site test devices. The main reason for this kind of approach was to guarantee that the police officers learn to use the on-site test devices in their duties, not the research scientists. In short, we took a longer perspective that the on-site testing, if acceptable, would not be only the part of the EU-project, but also an option for a police officer to handle drugs and driving cases in the future in Finland.

Subject selection. The testing was performed on drivers suspected to be under the influence of drugs, e.g., based on signs of erratic driving performance, which gave reason for initial suspicion to the police officer. In contrast to a few participating countries of ROSITA 2, there was no road blocks organised for testing the whole traffic flow.

Sample collection protocol and on-site testing. ROSITA 2 sample collection and on-site oral fluid test evaluation were based on existing legislative system in Finland. Only oral fluid samples were collected on a voluntary basis from the test subjects. The permission for sample collection was asked in a form of written consent (Appendix 3) and a short information sheet, which was given to the test subject. Police officers were instructed to perform Drugwipe 5 on roadside in a case of initial suspicion of drug use. Furthermore, Drugwipe Benzodiazepines was tested during the project if practically possible from an operational point of view of a police officer, even if Drugwipe 5 was positive for some drug(s). The Drugwipe tests were performed by wiping the tongue 5-10 times according to the manufacturer's instructions. The police officers were also instructed to request a blood sample for laboratory confirmation, even if the Drugwipe(s) was negative, but it was suspected by the officer that the driver would be intoxicated. According to prevailing instructions of handling the drugs & driving cases in Finland, the police officer observations were documented to the field sobriety observation sheet (Appendix 1) in each case (including the on-site screening test result), a physician made the clinical performance test (Appendix 2) and took whole blood and possibly also urine samples. Furthermore, an oral fluid sample was taken by a police officer with the Intercept device (Orasure Technologies) within a few minutes from whole blood sample collection. The Intercept test device was kept in mouth for 3 minutes after wiping between the lower teeth and cheek a few times according to the manufacturer's instructions. The oral fluid sample in Intercept buffer solution, whole blood (and possibly urine) were sent with the field sobriety observation sheet, the clinical performance test evaluation and, if obtained, consent form with permission to take an oral fluid sample to the Drug Research Unit / National Public Health Institute for confirmatory laboratory analysis.

Laboratory confirmation

In laboratory analysis, cut-off values agreed in the first ROSITA 2 meeting in Strasbourg 2003, where used for quantitative purposes. These cut-off values are shown in Table 1. Oral fluid concentrations were determined based on volume of pure oral fluid, not diluted oral fluid (i.e. oral fluid + Intercept buffer solution).

Table 1. Cut-off values used in confirmatory laboratory analyses (GC–MS).
Concentration of each substance is informed in ng/ml.

Substance ¹	Oral fluid	Blood
Amphetamine	25	20
Metamphetamine	25	20
MDMA	25	20
Δ^9 -THC	2	1
THCC	-	5
THC-OH	-	1
Cocaine	8	20
Benzoylecgonine	8	20
Morphine	20	10
Codeine	20	10
6-MAM	2	2
Diazepam	5	50
Nordazepam	5	50
Oxazepam	5	50
Temazepam	5	50
Lorazepam	5	10
Clonazepam	5	5
Alprazolam	5	5
(Zolpidem)	5	25
(Midazolam)	2	20
(Phenazepam)	2	20
(Nitrazepam)	5	20
(Methadone)	20	25
(Ethyl morphine)	20	10
(Fentanyl)	2	-
(Ecgonine methyl ester)	-	20
(Pholcodine)	5	10
(Buprenorphine)	4	0.5
(Norbuprenorphine)	4	1
(MDEA)	25	20
(MDA)	25	20
(BDB)	25	20
(MBDB)	25	20

¹ The compounds in parentheses are additional compounds analyzed in oral fluid. The cut-off values of these compounds were not determined in Strasbourg 2003 between different countries.

Method development. During the ROSITA 2 project several laboratory methods were developed for whole blood and oral fluid matrices. The method development and validation procedures included over 1 000 drug determinations. All these methods have been published in well-known scientific publications during 2004-2006 [3-6]. Emphasis was especially put on the development of large-scale identification, screening and quantification procedure of the most commonly abused drugs in oral fluid. All methods were fully validated and accredited according to EN ISO/IEC 17025 standards.

Oral fluid. For estimating the total amount of oral fluid collected with the Intercept oral fluid specimen collector, empty test tubes were weighed prior to centrifugation. After weighing the empty test tubes, Intercept tubes were placed in the empty test tubes for centrifugation (15 min, 2,000 g). After the centrifugation, the test tubes (including diluted oral fluid) were re-weighed and the amount of oral fluid was calculated by the following formula (1):

$$V_{OF} \approx w_2 - (w_1 + w_{Int,avg}) \quad (1)$$

, where

- V_{OF} = volume of collected oral fluid sample [μ l]
- w_2 = re-weighed test tube after centrifugation of the Intercept sample [mg]
- = empty test tube + diluted oral fluid (oral fluid + Intercept buffer solution)
- w_1 = weighed (empty) test tube before centrifugation [mg]
- $w_{int,avg}$ = average weight of Intercept buffer solution [mg]

It was assumed that the density of Intercept blue buffer was 1.0 g/cm³, in other words 1 g = 1 ml. The average volume (weight) of the Intercept buffer solution (n=6) had to be used, because of variability of buffer volume in a sample collector. The centrifuged and weighed samples were frozen and stored at -20 °C until analyses. The delay between oral fluid sampling and analyses varied from a few days to approximately two months.

All oral fluid samples were analysed by a single GC–MS method covering 30 drugs of abuse [5]. The method was especially developed for the analysis of ROSITA 2 and was fully validated. The procedure was also accredited according to EN ISO/IEC 17025 standards by the Finnish Accreditation Service (FINAS) and includes the quantitative analysis of amphetamine, methamphetamine and several designer drugs including MDMA (ecstasy), cocaine, benzoyl ecgonine, Δ^9 -THC, codeine, 6-monoacetyl morphine, methadone, morphine, pholcodine, buprenorphine, norbuprenorphine, 10 benzodiazepines and fentanyl.

All diluted oral fluid (oral fluid sample + blue Intercept buffer solution) was thawed, diluted to phosphate buffer solution including 12 internal standards (pH 4.1), centrifuged (5 min, 2,000 g), poured to SPE column and handled according to the referred paper [5]. The blank Intercept oral fluid was collected by a few Intercept test devices from laboratory personnel. The blank oral fluid was then centrifuged, frozen and used as a background matrix in standard preparation. This procedure should guarantee that the background of standard solutions is very similar to the authentic oral fluid samples. 950 μ l of the collected blank Intercept oral fluid (including blue buffer) was added to all standards (+ 50 μ l of analytical standard solution) and blanks. The standards and blanks were diluted with phosphate buffer solution and treated exactly like the authentic samples. An overview of oral fluid sample preparation is given in Figure 3. For more accurate description of, e.g., preparation of solutions, sample preparation and mass spectrometric analysis, see the referred paper.

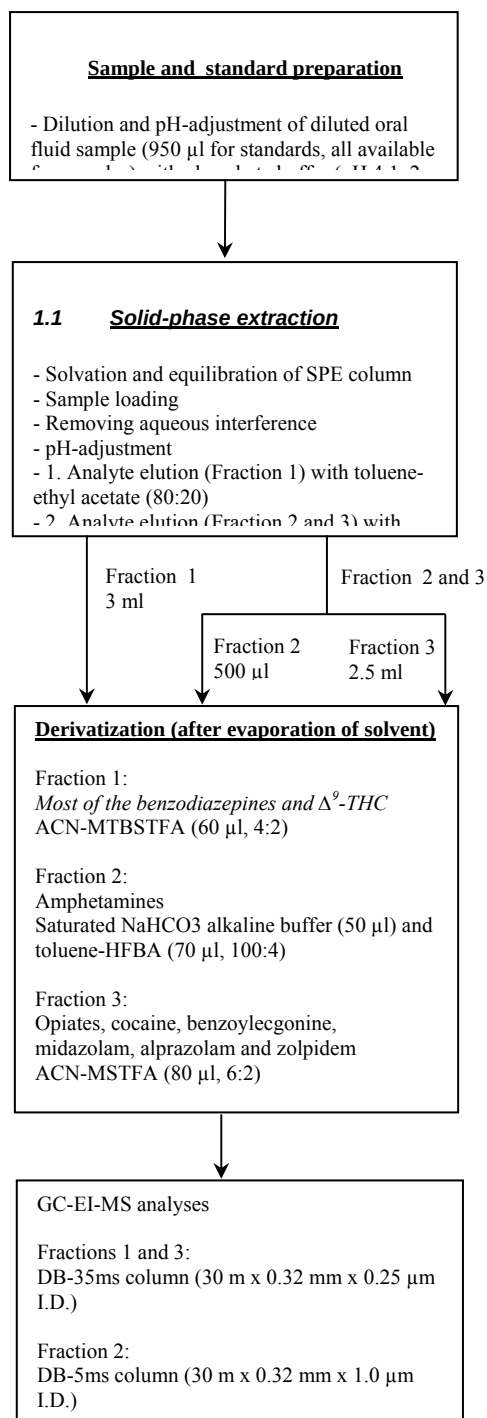


Figure 3. Overview of sample preparation

The quantitative results were calculated for all oral fluid samples taken into account the weighed amount of oral fluid in authentic samples. The standard calibrators were calculated to equal 250 µl of oral fluid. Consequently, if an authentic sample contained 375 µl or 125 µl of oral fluid, the final result was obtained by dividing the obtained GC-MS result with a factor of 1.5 or multiplying with a factor 2.0, respectively. Oral fluid

samples with less than 50 µl of oral fluid recovered were analysed and the results used for interpretation of positive or negative. The quantitative results for these samples were although disregarded in statistical calculations of the quantitative results. Analytical cut-off values agreed in the first ROSITA 2 meeting in Strasbourg 2003 were used (Table 1).

Whole blood. All whole blood samples were stored at +4 °C until analysis and were analysed according to the daily praxis at the laboratory. The substances hazardous or potentially hazardous to traffic safety were screened with immunological methods [7] as well as gas chromatography–mass spectrometry (GC–MS) and GC–ECD [3], and further confirmed by separate GC–MS methods [4,6,8,9] based on both electron impact (EI) and negative chemical ionization (NCI). For amphetamines, the same GC–MS method was used for screening and quantification [8]. All methods were validated and accredited according to EN ISO/IEC 17025 standards by the Finnish Accreditation Service (FINAS) and all quantification analyses on blood were performed in duplicates. Analytical cut-off values agreed in the first ROSITA 2 meeting in Strasbourg 2003 were used in quantitative analyses (Table 1).

Urine. Urine samples are not typically analysed anymore in drugs and driving cases in Finland after the introduction of zero tolerance law in February 2003, which is based on whole blood results. For ROSITA 2, however, the available urine samples were screened by immunological method. In addition, quantification analyses for positive cases of amphetamine group were performed by GC–MS [8] for comparing the concentrations of amphetamine in all collected biological specimens, i.e. whole blood, urine and oral fluid. Urine samples were stored at +4 °C until analysis.

Interpretation of the results and definitions

Results of the Drugwipe 5 and Benzodiazepines tests were compared to the GC–MS confirmation results. GC–MS result was considered positive if the result was above cut-off values agreed in Strasbourg 2003 (See Table 1). Definitions, such as true positive, false positive, false negative and true positive, as well as statistical characteristics, such as sensitivity, specificity and accuracy, are described in Appendix 4.

Feedback from police officers

Comments, experiences and evaluation of Drugwipe on-site test devices were systematically inquired from police officers after the testing period of ROSITA 2 in a form of questionnaire (Appendix 5). The questionnaire was kept short and simple consisting of 12 questions and two A4 pages mainly concentrating on practical aspects.

Results

Number of subjects and basic demographics

The total number of cases was 274. Most subjects were men, in all 82.8%. The median and average age for all was 31 and 32.3, respectively, in a range of 18-59 years. The distribution over age, females and males separately, is shown in Figure 4.



Figure 4. Demographics over participants (bar interval 5 years)

Practical evaluation of Drugwipe 5 and Benzodiazepines by the police officers

Police officers, who participated to the questionnaire organized, had performed 442 Drugwipe 5 and 246 Drugwipe Benzodiazepine tests (totally 688). 36 of these tests were failed, i.e. no control lines showed up. The number of Drugwipes performed by police officers participating to questionnaire was higher than in ROSITA 2 material since Finnish police has purchased Drugwipes on their own after the results from ROSITA 2 project.

It should be noted that the more experienced police officers were more satisfied with the features asked, such as the ease of use and the credibility of the test, interpretation of the result and the usability of the test for on-site testing. Furthermore, there were less test failures in percentages within the more experienced group. The result of the questionnaire in terms of practical failure rates of Drugwipe tests is given in Table 2. Police officers were divided to two groups: Group A included officers, who had performed over 10 Drugwipe tests, and Group B, who had performed less than 10 Drugwipe tests.

Table 2. The number of police officers participated in questionnaire, performed and failure rates of Drugwipe tests.

	Number of officers	Drugwipe 5			Drugwipe Benzodiazepines		
		Tests performed	Tests failed	Failure rate (%)	Tests performed	Tests failed	Failure rate (%)
Group A ¹	17	367	10	2.7	207	5	2.4
Group B ¹	31	75	17	22.7	37	4	10.8

¹Group A consists of officers who had performed 10 or more Drugwipe tests, Group B of officers who had performed less than 10 tests.

Some criticism was obtained from both groups about the interpretation of the results. Especially the results from the cannabis test are generally quite difficult to interpret. It should be noted, however, that all police officers were willing to continue with Drugwipe and also the testing time (evaluated 5-15 min) was considered acceptable by most of the officers when dealing with a person suspected of driving under the influence of drugs.

Descriptive statistics of the laboratory confirmation results

In all oral fluid samples (n=154), the volume of oral fluid was measured for quantitative purposes (*see Methods/Laboratory confirmation*). The collected amount of oral fluid varied significantly depending on the person tested. The average volume of measured oral fluid was only 0.224 µl with a minimum of 0.000 µl, maximum of 0.795 µl and relative standard deviation (RSD) of 103%.

The samples with less than 50 µl of oral fluid recovered, in all 39 cases, were analysed but the results were not included in the statistical interpretation of quantitative data. Furthermore, very high concentrations in several amphetamines, a few benzodiazepine cases in addition to one Δ⁹-THC case (1180 ng/ml) found in oral fluid must be regarded with caution, as the results exceed the linearity range. It must be noted that the oral fluid confirmation samples for laboratory analyses were obtained from the drivers on voluntary basis. Oral fluid samples were not available in 120 cases.

Blood samples were available in 273 cases. Three samples consisted only of a small amount of blood (1-2 ml). In these cases, whole blood sample collection had been extremely difficult because of a long history of intravenous substance abuse. In the authors' laboratory and in case of limited blood sample volume, the priority has been in the confirmation analyses of illicit drugs.

In all, abuse of illicit drugs (amphetamines, cocaine, heroin, cannabis) was confirmed in 94.5% (n=259) of the cases (n=274) in oral fluid and/or whole blood. Benzodiazepines and other medicinal drugs were found in 55.1% (n=151) of the cases (n=274). There were 13 cases in which only medicinal drugs were confirmed. Three cases could not be analyzed for medicinal drugs, because of insufficient sample volume. There were two cases that were completely negative.

Amphetamine-type stimulant drugs

The number of positive amphetamine, metamphetamine, MDMA, MDA and MDEA cases and respective concentration ranges are shown in Table 3.

Table 3. The number of positive amphetamine (AMP), metamphetamine (MAMP), MDMA, MDA and MDEA cases and respective concentrations ranges (ng/ml).

	AMP		MAMP		MDMA		MDA		MDEA	
	N	Range	N	Range	N	Range	N	Range	N	Range
Oral fluid	101 ^a	27.8-131000	5	28.5-63.9	4 ^a	29.2-12700	2	223-410	1	413 ^b
Blood	223	39.0-4030	1	861 ^b	12	31.5-340	1	28.0 ^a	2	40.0-57.0

^a In addition, there were 36 amphetamine and 3 MDMA positive samples, where accurate quantitative data was not calculated due to the insufficient oral volume (<50 µl).

^b Only one positive case was confirmed.

In all methamphetamine cases (28.5-63.9 ng/ml) in oral fluid, also high concentration of amphetamine was quantified (3 310-56 600 ng/ml) indicating that methamphetamine was only a synthesis impurity of amphetamine. Furthermore, in both MDA (220 and 410 ng/ml) positive cases in oral fluid also MDMA (3 030 and 12 700 ng/ml) was found indicating that MDA was only a metabolic product of MDMA.

MDMA was confirmed positive in all whole blood cases (96.0, 153 and 53.5 ng/ml) where oral fluid was MDMA positive (3 030, 12 700 ng/ml, in addition one sample contained high concentrations of MDMA, but accurate result was not calculated because of the limited sample volume, <50 µl, respectively). In addition, there were 4 MDMA positive samples (29.2-702 ng/ml) in oral fluid, which were confirmed negative in whole blood. 36 amphetamine and 3 MDMA oral fluid results were not considered accurately quantified due to the low volume of oral fluid sample (<50 µl). The results for confirmation analysis of amphetamine-type stimulant drugs in oral fluid and whole blood are shown in Figure 5 and Figure 6.

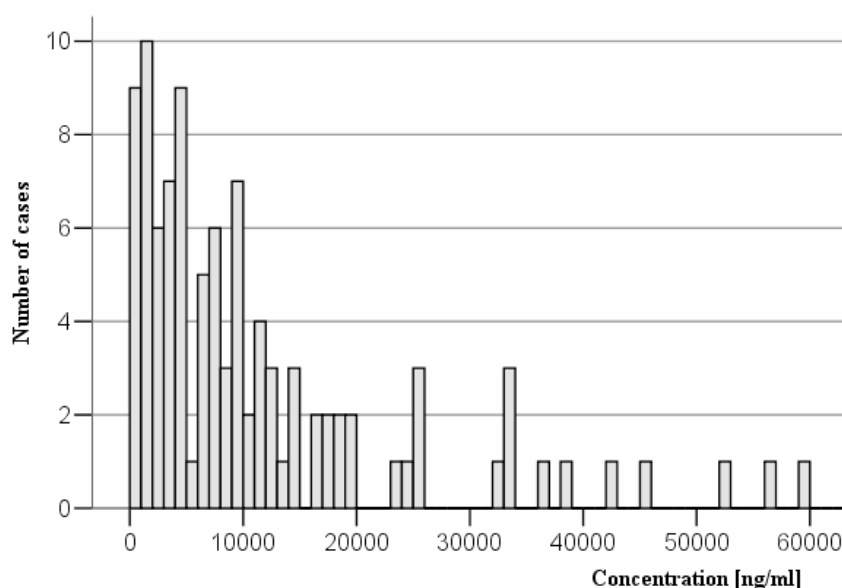


Figure 5. Results of confirmation analyses for amphetamine in oral fluid. Concentration frequencies with bar interval of 1000 ng/ml (cut-off 25 ng/ml). One amphetamine positive sample (131 000 ng/ml) was omitted from the figure.

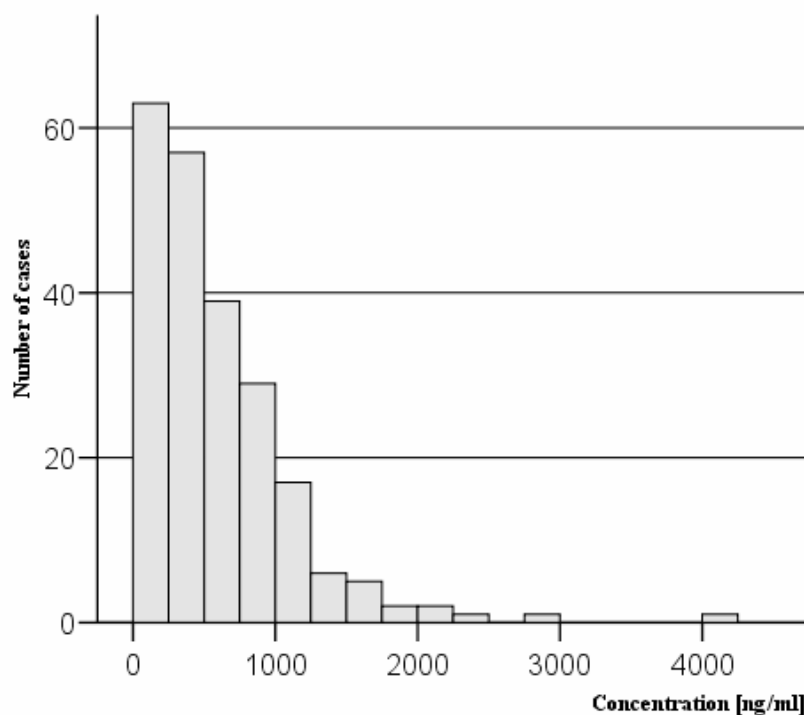


Figure 6. Results of confirmation analyses for amphetamine in whole blood. Concentration frequencies with bar interval of 250 ng/ml (cut-off 20 ng/ml).

Cannabis

There were 23 Δ^9 -THC positive oral fluid samples. The concentration range was 2.3-1180 ng/ml. In seven cases the oral fluid volume was insufficient for accurate quantification (oral fluid sample volume <50 μ l). The distribution of the Δ^9 -THC results for oral fluid is shown in Table 4.

Table 4. Occurrence of Δ^9 -THC in oral fluid, concentrations in ng/ml.

Number of quantified cases	Range	Mean	Median
16	2.30-1180	99.7	16.2

There were 41 blood samples positive for Δ^9 -THC. The blood confirmation results ranged between 1 and 11 ng/ml. The distribution of the confirmed results is shown in Figure 7. THCC was found in 77 blood samples. Concentrations varied between 5.20 and 92.3 ng/ml. The distribution of positive THCC in whole blood samples is shown in Figure 8.

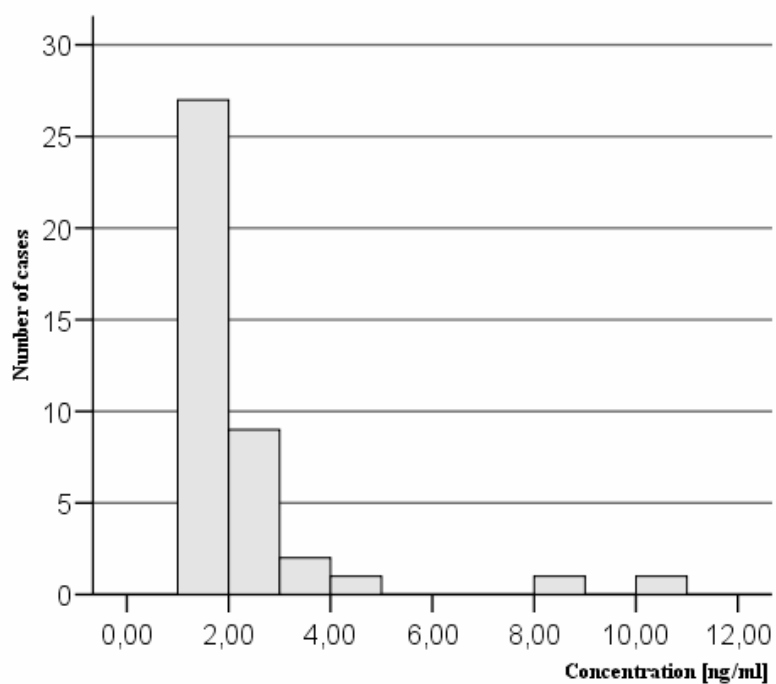


Figure 7. Distribution of concentration of Δ^9 -THC positive in whole blood samples , bar interval 1 ng/ml, cut-off 1 ng/ml.

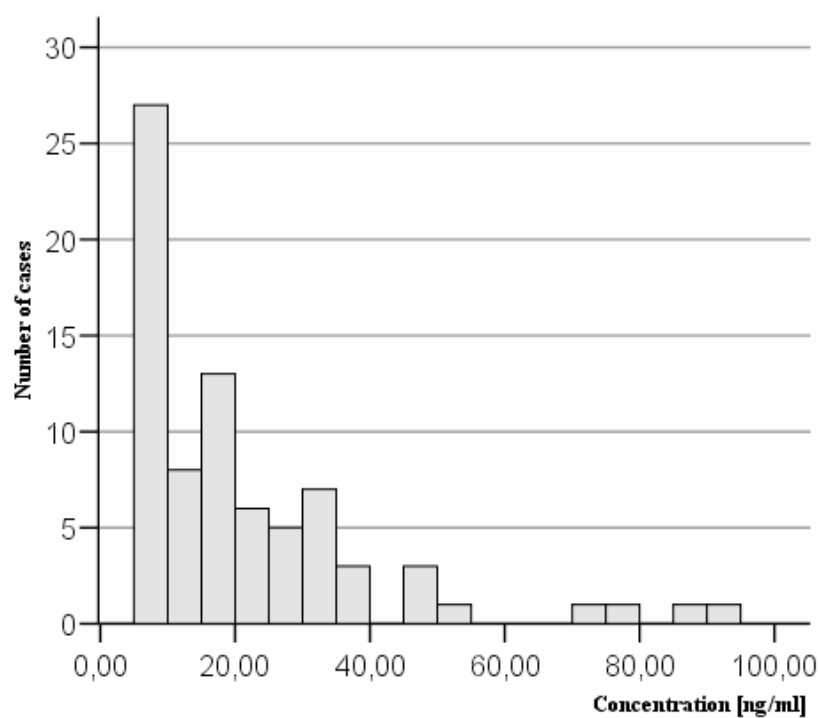


Figure 8. Distribution of concentration of positive Δ^9 -THCC results in whole blood samples, bar interval 5 ng/ml blood and cut-off 5 ng/ml.

Cocaine

Two oral fluid samples were confirmed positive for cocaine. The first one contained 10.3 ng/ml of cocaine and 53.3 ng/ml of benzoylecgonine. In the second case the volume of

the oral fluid sample was insufficient for accurate quantitation, but both cocaine and benzoylecgonine were confirmed positive. Blood confirmation of both of the cases was negative for cocaine, but 39.5 ng/ml of benzoylecgonine was confirmed in the first case and 300 ng/ml in the second case. There were also two cases in which no oral fluid sample was available. In the first case 98.5 ng/ml of cocaine, 332 ng/ml of benzoylecgonine and 33 ng/ml of ecgonine methyl ester were confirmed in blood. In the second case 384 ng/ml of benzoylecgonine was confirmed in blood.

Opiates

Two oral fluid samples were confirmed positive for morphine. Morphine concentration of the first case was 22.5 ng/ml. In the second case also 6-MAM was confirmed. The concentrations were 50.7 ng/ml for morphine and 9.1 ng/ml for 6-MAM. No morphine was confirmed in blood in these cases. One blood sample was confirmed morphine and 6-MAM positive. The concentrations were 14.3 ng/ml and 6.3 ng/ml, respectively.

Codeine was confirmed in seven blood samples. In two of these cases codeine was also confirmed in oral fluid. In four cases no oral fluid sample was available. In one case codeine was confirmed only in the oral fluid sample. Codeine results are shown in Table 5.

Table 5. Confirmation results for codeine. Concentrations in ng/ml, n.d. = not detected, no OF = no oral fluid sample was available, + = oral fluid volume insufficient for accurate quantitation (<50 µl).

Case	Blood	Oral fluid
12	115	905
73	46.0	no OF
82	10.0	110
164	14.0	n.d.
196	n.d.	+
200	37.0	no OF
237	74.5	no OF
247	25.5	no OF

Benzodiazepines

There were all in all 64 benzodiazepine positive oral fluid samples and 136 benzodiazepine positive whole blood samples. The number of positive and negative cases is shown in Table 6. Benzodiazepine findings in oral fluid and blood are shown in Table 7 and Table 8.

Table 6. The number of positive and negative benzodiazepine cases based on the oral fluid and blood samples.

	Oral fluid		Blood	
	N of cases	% of cases	N of cases	% of cases
Positive	64 ^a	41.6	136	49.8
Negative	90	58.4	137	50.2
Total	154	100	273	100

^a In 18 cases the oral fluid sample volume was below 50 µl. These samples were not included in accurate quantitative calculations based on oral fluid drug concentrations (see Table 7).

Table 7. Benzodiazepine findings in oral fluid samples. Concentration range, mean and median reported in ng/ml.

Benzodiazepine	N of positive cases	Range	Mean	Median
Diazepam	22	6.2-62.5	29.7	24.4
Oxazepam	11	6.1-31.8	17.5	16.2
Nordazepam	30	5.2-76.5	18.0	13.1
Temazepam	8	7.2-28800	3642	25.8
Alprazolam	2	19.2-35.7	27.5	27.5
Lorazepam	1	54.6	54.6	54.6
Clonazepam	1	288	288	288
Nitrazepam	1	1090	1090	1090
Midazolam	1	134	134	134

Table 8. Benzodiazepine findings in blood samples. Concentration range, mean and median reported in ng/ml.

Benzodiazepine	N of positive cases	Range	Mean	Median
Diazepam	91	54.5-2500	427	292
Oxazepam	24	50.0-5280	451	153
Nordazepam	105	50.0-2020	357	257
Temazepam	30	50.8-6490	679	294
Alprazolam	25	12.0-208	61.2	44.0
Lorazepam	2	19.0-44.4	31.7	31.7
Clonazepam	12	15.0-87.0	39.8	29.5
Nitrazepam	5	29.0-110	70.5	79.5
Midazolam	1	332	332	332

Evaluation of the Drugwipe test devices compared with oral fluid and whole blood

A total of 268 people suspected of driving under the influence of drugs were tested with the Drugwipe 5 test (n=146) or both Drugwipe 5 and Drugwipe Benzodiazepines tests (n=121). In one case only Drugwipe Benzodiazepines test was performed. A few other cases were also tested with Drugwipes by a police officer, but because of unclear and/or insufficient documentation of Drugwipe results, these cases were omitted from the evaluations of the Drugwipes. Furthermore, sometimes when a positive result was obtained for illicit drugs with the Drugwipe 5 test, the test for benzodiazepine use was not performed, even though the instructions for police officers during the ROSITA 2 testing period were to perform both tests. For practical reasons, e.g., in a case of hurry and/or difficult person, the benzodiazepine test might have been omitted by a police officer.

The results of the Drugwipe tests were compared with the oral fluid and whole blood GC-MS confirmation results. Three different evaluations were made. The results were compared with the findings in 1) oral fluid sample; 2) whole blood sample, and 3) oral fluid sample, and if oral fluid was not available, to the findings in whole blood. A summary of the results is shown in Appendix 6.

Amphetamine-type stimulant drugs

The device evaluation for Drugwipe 5 amphetamine test based on oral fluid GC–MS confirmation results, whole blood GC–MS results and both GC–MS results are shown in Table 9.

Table 9. Device evaluation for amphetamine.

	Oral fluid	Blood	Oral fluid and blood
TP	128	216	218
FP	1	6	5
FN	6	5	11
TN	13	39	33
Total	148	266	267
Sensitivity	95.5%	97.7%	95.2%
Specificity	92.9%	86.7%	86.8%
Accuracy	95.3%	95.9%	94.0%
PPV	99.2%	97.3%	97.8%
NPV	68.4%	88.6%	75.0%
Prevalence	90.5%	83.1%	85.8%

False positive cases. One oral fluid sample that tested positive with the Drugwipe 5 device did not contain any amphetamine or amphetamine-related substances in oral fluid. Furthermore, no ATSS were confirmed in whole blood.

Six false positive Drugwipe results were obtained when using whole blood matrix for comparison. One sample included pseudoephedrine (385 ng/ml), which have might cross-reacted with amphetamine group antibody. Furthermore, in one sample low concentration of amphetamine was found in oral fluid (151 ng/ml), which was Drugwipe positive. Other samples were completely negative in whole blood and/or oral fluid.

False negative cases. There were six false negative cases found based on the oral fluid GC–MS confirmation results. The concentrations of amphetamine in oral fluid in five false negative cases varied between 27.8-1230 ng/ml. MDMA was confirmed in two samples, but no exact quantitative result was calculated due to the low amount of oral fluid (<50 µl). The other sample included only MDMA. There was no metamphetamine found in any of the false negative cases. It should be noted that false negative amphetamine and MDMA concentrations are pretty low in a comparison to average concentrations found (See *descriptive statistics of the confirmation results*). It should also be noted that all amphetamines positive in oral fluid were also positive in whole blood. The false negative cases are listed in Table 10.

Table 10. Amphetamine and MDMA concentrations in oral fluid in false negative cases. Concentrations in ng/ml, n.d.=not detected, +=positive, but insufficient volume of oral fluid for accurate quantitation (<50 µl).

Case	Amphetamine	MDMA
30	1230	n.d.
46	+	+
69	n.d.	+
124	160	n.d.
147	144	n.d.
219	27.8	n.d.

Amphetamine along with cannabis is the most frequently found drug among drugged drivers in Finland [1]. This is also demonstrated by the high prevalence (90.5% based on oral fluid confirmation results and 83.1 % based on blood confirmation results) of amphetamine obtained in this study. Since there were a high number of true positive results, a reliable evaluation for the sensitivity of the Drugwipe 5 device for amphetamine was obtained. Sensitivity, specificity and accuracy for amphetamine screening with Drugwipe calculated based on oral fluid confirmation results were 95.5%, 92.9% and 95.3% respectively.

Cannabis

Device evaluation results for Drugwipe 5 cannabis test are presented in Table 11.

Table 11. Results for cannabis.

	Oral fluid	Blood	Oral fluid and blood
TP	12	28	29
FP	11	27	26
FN	11	13	18
TN	114	197	193
Total	148	265	266 ¹
Sensitivity	52.2%	68.3%	61.7%
Specificity	91.2%	87.9%	88.1%
Accuracy	85.1%	84.9%	83.5%
PPV	52.2%	50.9%	52.7%
NPV	91.2%	93.8%	91.5%
Prevalence	15.5%	15.5%	17.7%

¹ One Δ^9 -THC positive screening test could not be confirmed due to the limited volume of whole blood (no oral fluid was available). In addition, the test was opiate and amphetamine positive, which were both confirmed positive by GC–MS.

Δ^9 -THC concentrations in cases in which Drugwipe cannabis result was positive were in the range of 6.5-1178.5 ng/ml.

False positive cases. There were 11 cases in which cannabis was positive with Drugwipe but no Δ^9 -THC could be confirmed in oral fluid by GC–MS. In six cases Δ^9 -THC and/or THCC was confirmed in the blood sample. In both Δ^9 -THC positive whole blood cases, the volume of oral fluid was very low (<0.075 μ l). Results for the false positive cases are listed in Table 12.

Table 12. False positive cannabis cases. Concentrations in ng/ml, n.d. = not detected.

Case	Δ^9 -THC oral fluid	Δ^9 -THC blood	THCC blood
48	n.d.	n.d.	3.10
70	n.d.	n.d.	n.d.
82	n.d.	n.d.	n.d.
89	n.d.	n.d.	9.70
90	n.d.	n.d.	n.d.
95	n.d.	1.60	48.0
103	n.d.	n.d.	n.d.
122	(1.40)	n.d.	32.0
164	n.d.	n.d.	5.40
191	n.d.	1.70	28.0
242	n.d.	n.d.	n.d.

[†] Δ^9 -THC was below the agreed cut-off value (2 ng/ml, see Table 1).

In addition to lack of specificity of Δ^9 -THC antibody, a few other factors should be considered as a source of false positive results. First, the cannabis line in Drugwipe 5 test is usually very weak, even if the test is true positive. The interpretation of a weak test lines increases the risk of false positives, even though it might increase the sensitivity of the test. And second, the adsorption of Δ^9 -THC onto the Intercept specimen collector and degradation during storage before GC–MS confirmation might affect to laboratory results. The former phenomenon has already been demonstrated with other collection devices [10]. It has also been shown that only approximately 50 % of Δ^9 -THC can be recovered from the Intercept device and that a decrease of 20 % in Δ^9 -THC concentration is likely during six week storage at -20°C [11].

False negative cases. There were 11 cases in which cannabis was found negative with Drugwipe but concentrations above the Strasbourg cut-offs were confirmed in oral fluid. Confirmed concentrations were in the range of 2.3-48.4 ng/ml. The cases are shown in Table 13.

Table 13. False negative cannabis cases based on oral fluid confirmation results. Concentrations in ng/ml, n.d. = not detected, + = insufficient volume of oral fluid for accurate quantitation (<50 μ l).

Case	Δ^9 -THC oral fluid	Δ^9 -THC blood	THCC blood
26	2.30	n.d.	2.40
62	8.30	n.d.	3.50
67	16.8	n.d.	6.90
85	4.10	n.d.	6.00
86	3.40	n.d.	n.d.
100	2.60	n.d.	9.00
109	+	n.d.	4.10
156	48.4	2.20	30.7
196	+	n.d.	3.20
246	15.5	1.15	5.40
258	23.3	2.00	18.8

Cocaine

Evaluation of the Drugwipe 5 results for cocaine is shown in Table 14.

Table 14. Cocaine results.

	Oral fluid	Blood	Oral fluid and blood
TP	1	2	2
FP	1	4	4
FN	1	2	2
TN	145	258	259
Total	148	266	267
Sensitivity	50.0%	50.0%	50.0%
Specificity	99.3%	98.5%	98.5%
Accuracy	98.6%	97.7%	97.8%
PPV	50.0%	33.3%	33.3%
NPV	99.3%	99.2%	99.2%
Prevalence	1.4%	1.5%	1.5%

One false positive case and one false negative case were found. In the false negative case 10.3 ng/ml of cocaine and 53.3 ng/ml of benzoylecgonine were confirmed with GC–MS in oral fluid. Benzoylecgonine was also found in blood in this case (concentration 39.5 ng/ml). Since there was only one true positive result, the low value for sensitivity (50.0 % according to every calculation) obtained in this study should be regarded with caution. The specificity of the test was acceptable (99.3%), when the evaluation was based on the oral fluid.

Opiates

The evaluation of the performance of Drugwipe 5 for detecting opiates is presented in Table 15.

Table 15. Results for opiates.

	Oral fluid	Blood	Oral fluid and blood
TP	4	7	8
FP	6	8	7
FN	0	1	1
TN	138	250	251
Total	148	266	267
Sensitivity	100%	87.5%	88.9%
Specificity	95.8%	96.9%	97.3%
Accuracy	95.9%	96.6%	97.0%
PPV	40.0%	46.7%	53.3%
NPV	100%	99.6%	99.6%
Prevalence	2.70%	3.00%	3.40%

The evaluation was made based on morphine, codeine and 6-MAM GC–MS confirmation results. Pholcodine, buprenorphine and norbuprenorphine were also confirmed by GC–

MS. Pholcodine did not exist in any sample, and buprenorphine and norbuprenorphine (n=8) results were considered as opiate negative, since the manufacturer (Securetec) has informed that the device cannot detect buprenorphine and/or its metabolite.

Five false positive cases were found based on the results of oral fluid GC–MS confirmation. No morphine, 6-MAM or codeine were confirmed in these cases either in oral fluid or blood except for one case, in which a low concentration of codeine (14.0 ng/ml) was confirmed in the blood sample. At this point, it should be noted that one sample gave positive test result for opiates, but no morphine, codeine and/or 6-MAM was not detected in oral fluid. Nevertheless, very high concentration of buprenorphine (1480 ng/ml; norbuprenorphine 6.9 ng/ml) was detected in oral fluid indicating possible contamination of oral cavity after a recent oral intake of Subutex®. On the basis of this limited data, it might be possible that Drugwipe 5 opiate antibody has also some cross-reactivity to other morphine-like core structures in addition to morphine, codeine and 6-MAM, such as buprenorphine. There were no false negative cases.

Benzodiazepines

Evaluation of the performance of Drugwipe Benzodiazepines test is shown in Table 16.

Table 16. Evaluation for Drugwipe Benzodiazepines results for benzodiazepine screening.

	Oral fluid	Blood	Oral fluid and blood
TP	29	50	48
FP	6	6	9
FN	10	25	19
TN	32	40	46
Total	77	121	122
Sensitivity	74.4%	66.7%	71.6%
Specificity	84.2%	87.0%	83.6%
Accuracy	79.2%	74.4%	77.0%
PPV	82.9%	89.3%	84.2%
NPV	76.2%	61.5%	70.8%
Prevalence	50.6%	62.0%	54.9%

False positive cases. There were six cases confirmed false positive according to the results of oral fluid GC–MS confirmation. Nevertheless, in two cases benzodiazepines were detected below cut-off value. The other sample contained 2.5 ng/ml and 4.0 ng/ml of diazepam and nordazepam, respectively (respective whole blood concentrations were 120 and 100 ng/ml), and the other sample 4.7 ng/ml of nordazepam (whole blood concentrations were 90 ng/ml of diazepam and 46 ng/ml of nordazepam).

False negative cases. Ten false negative cases were found based on oral fluid confirmation results. The volume of the oral fluid sample was insufficient for accurate quantitation in seven of the false positive cases. Benzodiazepine results for false negative oral fluid samples are shown in Table 17.

Table 17. Oral fluid GC–MS confirmation results of the false negative benzodiazepine cases. Concentrations in ng/ml, n.d.=not detected, += insufficient volume of oral fluid for accurate quantitation (<50 µl).

Case	Diazepam	Nordazepam	Oxazepam	Temazepam	Alprazolam
13	n.d.	n.d.	n.d.	n.d.	+
42	n.d.	8.00	n.d.	n.d.	n.d.
51	n.d.	n.d.	n.d.	+	n.d.
92	+	+	n.d.	n.d.	n.d.
109	n.d.	+	n.d.	n.d.	n.d.
176	+	+	n.d.	+	n.d.
180	n.d.	n.d.	n.d.	177	n.d.
196	+	+	+	n.d.	n.d.
205	51.8	36.5	n.d.	n.d.	n.d.
236	+	+	n.d.	+	n.d.
Total	5	7	1	4	1

Benzodiazepines are a complicated group for on-site testing in oral fluid. The concentrations of benzodiazepines are generally much lower in oral fluid than in whole blood or urine. Especially the concentrations of low-dosed benzodiazepines are generally at low ng/ml or even below in oral fluid. In addition, cross-reactivity of an antibody might vary a lot depending on the substance. In addition, cross-reactivity with other medicines has been reported earlier with urine on-site tests [12]. Sensitivity and specificity calculated from the oral fluid GC–MS results were 74.4% and 84.2%, respectively.

Evaluation of the Drugwipe test devices compared to urine immunoassay

There were also 144 cases in which a urine sample was available. The urine samples were screened with EMIT immunoassay for amphetamines, cannabis, cocaine, opiates and benzodiazepines, and the results were compared with Drugwipe test results. The results of the evaluation are shown in Table 18.

Table 18. Device evaluation based on urine screening results.

	Amphetamine	Cannabis	Cocaine	Opiates	Benzodiazepines
TP	119	24	2	8	31
FP	3	3	2	1	2
FN	7	55	0	8	23
TN	15	62	140	127	9
total	144	144	144	144	65
sensitivity	94.4%	30.4%	100%	50.0%	57.4%
specificity	83.3%	95.4%	98.6%	99.2%	81.8%
accuracy	93.1%	59.7%	98.6%	93.8%	61.5%
PPV	97.5%	88.9%	50.0%	88.9%	93.9%
NPV	68.2%	53.0%	100%	94.1%	28.1%
prevalence	87.5%	54.9%	1.4%	11.1%	83.1%

The comparison between urine immunological screening and on-site test results (Drugwipe) is quite different from the results obtained when the evaluation was made based on oral fluid or blood GC–MS confirmation results. Prevalence values for cannabis, opiates and benzodiazepines are clearly increased due to the longer detection times in urine than in oral fluid or whole blood. There is also some distortion seen in sensitivity, specificity and accuracy values obtained by using urine, instead of whole blood and/or oral fluid.

Discussion

Practical aspects

In Finland, the common interest of authorities, police officers and research scientists in addition to supportive public opinion create a convenient starting point for any improvements of handling the drugs and driving cases. In the beginning of the ROSITA 2 project, the police officers were, in most cases, very eager to participate in roadside drug testing and to learn how to use on-site drug testing devices. At the end of this project, the comments were still positive. Most of the police officers see the on-site drug testing as a powerful tool for identification and as additional evidence for their suspicion of drug abuse. Furthermore, many suspects have admitted the substance abuse even before the police officer has started to perform the Drugwipe test, during the testing or immediately after a positive result. On-site screening tests might, even though hard to document, have a preventive influence on drugged driving.

It should be emphasized the importance of size, practicability, rapidity and portability of testing device from the operational point of view. Police officers have been satisfied with the practical considerations of Drugwipe, because the device fulfils at least partially these requirements. It has been also very practical that devices can be delivered to different policemen and they can even put them into the pocket. Even the testing time, which was evaluated to be 5-15 minutes, was not considered too long time by most of the officers. It should be noted that many police officers have reported that the detection of cannabis takes generally much longer time than the other drugs (10-15 min). Thin red colour of cannabis lines (indicating positive test) also carries a risk of false positive case, which can also be noted from the comparisons between Drugwipe and laboratory confirmatory results. There were too many false positive cannabis cases. In addition to thin lines of the test and sometimes difficult interpretation of the test result, practical problems have been noted with, e.g., using cold water for testing and/or test devices at winter time. Furthermore, the importance of education of police officers should not be underestimated. The police officers with more experience of using Drugwipe were generally more satisfied with the device.

Analytical evaluation of the roadside devices

Based on the evaluation between Drugwipe 5 and Drugwipe Benzodiazepine tests performed at roadside, and laboratory confirmatory results analyzed by GC–MS, the applicability of Drugwipe to detect different drugs is highly variable. The sensitivity, specificity and accuracy confirm that the screening result obtained with the Drugwipe 5 test correlate quite well with amphetamine oral fluid GC–MS confirmation results. An inferior correlation is obtained for cocaine and opiate results. Yet one must bear in mind that due to the low number of positive opiate and cocaine cases, much cannot be said

about the sensitivity of the test for these drug classes. At the same time the high number of true negative cases makes the specificity evaluations for these substances quite reliable. Theoretically, both cocaine and opiates should be more easily detected in oral fluid because of generally higher concentrations compared with Δ^9 -THC and benzodiazepines.

The results obtained for cannabis (Δ^9 -THC) detection, however, were unsatisfactory. The sensitivity obtained (52.2 % calculated based on oral fluid GC–MS confirmation results) is unacceptable for an on-site device. A lot of development work must be done in order to achieve acceptable sensitivity in cannabis on-site testing. Kintz et al. have suggested that on-site screening devices should be able to give positive response for Δ^9 -THC at the level of 2 ng/ml [10]. Based on this study, it seems that the suggested cut-off level is still unattainable.

The relatively high prevalence of benzodiazepines (50.6% calculated from the oral fluid confirmation results) demonstrates a need for a sensitive, specific and accurate on-site benzodiazepine testing device for detecting drugged drivers in Finland. Based on the data, the sensitivity of Drugwipe Benzodiazepines should still be improved. However, the device worked quite well, when taking into account the low concentration levels of benzodiazepines in oral fluid. Lately, the manufacturer announced that they have succeeded in lowering the cut-off value from 10 ng/ml to 5 ng/ml. Moreover, in many benzodiazepine positive cases also metabolic products, e.g., nordazepam were confirmed by GC–MS. This should ease the identification of benzodiazepines by on-site testing devices based on immunological techniques.

Interestingly, the sensitivity, specificity and accuracy evaluations made based on oral fluid GC–MS confirmation results, whole blood GC–MS confirmation results and results from both matrices are quite uniform. This further indicates that oral fluid is a suitable matrix for initial screening tests at the roadside and the results seem to correlate with oral fluid and whole blood (which is used in confirmatory analysis in Finland).

Oral fluid sample collection for confirmatory analysis

As noted by Crouch [11] oral fluid collection seems to be the neglected variable in oral fluid testing. Low volume and wide variability of oral fluid collected with the Intercept device (average 0.224 μ l, RSD 103%) further evidence the problems confronted with the oral fluid collection. In laboratory conditions, however, much higher volumes were collected (500-700 μ l), but the collection of oral fluid might be much more difficult, e.g., from amphetamine abusers, whose oral fluid secretion has been lowered [13].

The compounds are diluted with blue buffer in the Intercept device, which complicates the quantitative determination, especially because of the fact that the amount of buffer solution was not constant. Therefore, we had to use average volume to estimate the volume of oral fluid for quantitative purposes. Furthermore, there are some non-volatile components in blue Intercept buffer which might co-extract with the analytes, increase the background interference in GC–MS analysis and decrease the column life [5]. The problems with the buffer have also been encountered with LC/MS/MS in a form of ion suppression [14]. Further problems might be encountered, e.g., with oral contamination, drug stability in blue buffer and absorption of drugs in a cotton roll of specimen collector. Nevertheless, oral fluid specimen collectors (such as Intercept) are valuable and much

needed tools for collecting oral fluid in a more appropriate way than spitting. Furthermore, the limitations of the collection and problems in the laboratory should be carefully evaluated by the manufacturer.

Conclusion

Most of the police officers experience that oral fluid on-site test devices are valuable tools for helping with the identification and confirmation of initial suspicion of drug use. Furthermore, operating police officers have been very satisfied with the Drugwipe tests because of practicability for road side testing, i.e., ease of use, small size and sufficiently fast results. The comparison of laboratory confirmation results and Drugwipe results, however, indicate a significant variation of performance depending on the group of the drugs detected. Nevertheless, amphetamine and benzodiazepines, which are the most commonly found drugs in road traffic in Finland, had acceptable sensitivity and specificity in Drugwipe devices especially when high abused concentrations were found, even though the benzodiazepine test should still be further developed for being able to detect even lower concentrations of especially low-dosed benzodiazepines, e.g., alprazolam, clonazepam and lorazepam. The main problem with Drugwipe 5 test, however, was insufficient sensitivity and specificity for cannabis. This indicates that serious emphasis should be put on to the development of more sensitive and specific antibody for Δ^9 -THC by the manufacturer.

In the authors' opinion, there is no doubt that oral fluid as a biological matrix is the best alternative for initial screening tests at roadside. Easy, supervised and non-invasive sample collection in a combination with an indication of recent drug use, current pharmacological effects of a substance and detection windows resembling those of whole blood matrix will further increase the interest of oral fluid in toxicological analysis and on-site drug testing. In this study, very similar drug findings in oral fluid and whole blood were confirmed (unlike urine), which can be considered a further evidence of applicability of oral fluid for estimating the current intoxication of the person tested.

In the future, an interesting alternative would be to replace whole blood with oral fluid matrix also in confirmatory laboratory analysis. Especially in the countries where zero-tolerance law is applied for illicit drugs (as in Finland), oral fluid would be an interesting matrix since impairment of a person does not have to be shown in the court. Based on the Finnish part of ROSITA 2 project, to establish universally accepted cut-off values more work is still needed, not only to measure whole blood and oral fluid concentrations of the same person at the same time, but to invest time in oral fluid sample collection. Sample collection is among the most challenging obstacles in the way of using oral fluid instead of whole blood in routine confirmatory laboratory analyses.

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Appendices

- Appendix 1. Standardized field sobriety observation sheet
- Appendix 2. Documentation sheet of clinical performance test
- Appendix 3. Written consent for oral fluid collection
- Appendix 4. Definitions used in the evaluation of the Drugwipe results
- Appendix 5. Questionnaire for police officers about Drugwipe and on-site testing
- Appendix 6. Drugwipe device evaluations for each drug group



STANDARDIZED FIELD SOBRIETY OBSERVATION SHEET

Date

Concerns R-report nr.	Laboratory sample nr (filled out by KTL)
Surname and initial names	Social security nr.

OBSERVATIONS REGARDING WAY OF DRIVING, WEATHER AND ROADWAY

Way of driving

☐ No own observations	☐ Secure	☐ Unsteady	☐ Inappropriate speed	☐ Violation of way of priority
☐ Winding, deviation from straight line up to _____ meters.				
Number of deviations: _____ on a _____ meters of observation				
☐ Other attentions				

Control of devices of vehicle

☐ Driving with low revolutions	☐ Insecure use of gears	☐ Roaring of motor
☐ Other		

Fault and defects of vehicle

☐ No	☐ Yes, what?
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Weather and lighting

☐ Rain	☐ Hard wind / storm	☐ Snow / sleet	☐ Fog	☐ Daylight	☐ Dusk	☐ Dark
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Roadway

☐ Good	☐ Poor	☐ Construction on way	☐ Good lighting	☐ Poor lighting	☐ Dry	☐ Wet	☐ Icy / snowy
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OBSERVATIONS DURING STOPPING AND CONFRONTING

Reactivity

☐ Normal	☐ Slow	☐ Very slow	☐ None	☐ Sweating	☐ Tremor	☐ Vomiting	☐ Restlessness
---------------------------------	-------------------------------	------------------------------------	-------------------------------	-----------------------------------	---------------------------------	-----------------------------------	---------------------------------------

Appearance

☐ Neat	☐ Shabby	☐ Filthy	☐ Yes	☐ No	☐ Faltering
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Speaks Finnish or Swedish

Speech

☐ Clear	☐ Sputtering	☐ Thick	☐ Lipping
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Communication, sense of time and place

☐ Clear sense of time and place	☐ Drowsy	☐ Wakes up	☐ Deep sleep / unconscious	☐ Altered
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Behaviour

☐ At ease, behaved	☐ Agitated	☐ Aggressive	☐ Matey	☐ Frivolous	☐ Uninterested	☐ Defiant	☐ Weepy
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Rising out of vehicle

☐ Normal	☐ Balance disturbed	☐ Has to lean on vehicle
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Walking

☐ Secure	☐ Dragging	☐ Wobbly	☐ Balance disturbed
---------------------------------	-----------------------------------	---------------------------------	--

Smell of alcohol

☐ Yes	☐ No
------------------------------	-----------------------------

Alcometer test

☐ Yes: time _____ ‰	☐ No	☐ Cannot be done	☐ Refused
--	-----------------------------	---	----------------------------------

Positive on site tests

Cozart	Time	☐ Amphetamine	☐ Opiates	☐ Benzodiazepines	☐ Cocaine	☐ THC
Drugwipe	Time	☐ Amphetamine	☐ Opiates	☐ Benzodiazepines	☐ Cocaine	☐ THC
Other, what?		☐ Amphetamine	☐ Opiates	☐ Benzodiazepines	☐ Cocaine	☐ THC

Eyes

☐ Nothing abnormal	☐ Conjunctivas reddish	☐ Watery / gleaming	☐ Restless
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Pupils

☐ Normal	☐ Dilated	☐ Contracted	Reaction to light	☐ Slow	☐ Fast
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Nystagmus

☐ Jerky movement	☐ No jerking observed
---	--

Right about _____ mm Left about _____ mm

Lighting conditions on test site

☐ Daylight	☐ Dusk	☐ Night, streetlights	☐ Night, indoor
☐ Other, what?			

Conspicuous behaviour

☐ Did not change during evaluation	☐ Increased during evaluation	☐ Decreased during evaluation
---	--	--

Test started: time _____ Test ended _____

The ability of the driver

☐ Is not impaired	☐ Is impaired	☐ Is considerably impaired
--	--------------------------------------	---

Further information: like other observations, confiscated substances, pills, paraphernalia etc.

Time and place	Signature and name of observer
----------------	--------------------------------

Documentation sheet of clinical performance test

Inquirer of the examination.....

Examination place

Name of the subject Social security number

Proving of identity ☐ proved by the police ☐ other

The reason for examination ☐ drunken driving ☐ other felony ☐ other Wanted examination ☐ blood sample ☐ urine sample ☐ clinical examination

According to the subject ☐ none ☐ yes What? ☐ no answer Diseases ☐ none ☐ yes What? Blood pressure/..... mmHg Pulse/min Observed symptoms ☐ none ☐ yes What?

Observed injuries ☐ none ☐ yes What? Liquid treatment of the injured ☐ no ☐ yes What, how much?

Drugs and medication. Has the subject used these before or after the incident? What, when, how much? Injection marks ☐ no ☐ yes According to ☐ none ☐ yes regularly ☐ yes occasionally ☐ no answer The subject

On-site test ☐ saliva ☐ urine ☐ neg. ☐ pos. What? Alcohol breath testo/oo

Clinical examination Weight kg ☐ weighed ☐ given Body structure ☐ normal ☐ slim ☐ obese Height cm ☐ measured ☐ given

Examinations, observations	normal	slightly deviating	clearly deviating (underline the observation)
Consciousness	☐		☐ numb, sleepy, almost unconscious
Aware of the date and time, memory	☐	☐	☐
Walking straight forward	☐	☐	☐
Full turn while walking	☐	☐	☐
Romberg's test with eyes closed	☐	☐	☐
Finger to finger test	☐	☐	☐
Pulling oneself together			☐ observed
Behaviour	☐		☐ uninhibited, aggressive, angry, talkative, arrogant, unresponsive, limp, absentminded
Speech	☐		☐ inarticulate, spluttering, thick, faltering
Train of thought	☐		☐ illogical, jumpy, muddled
Mood	☐		☐ euphoric, irritated, distressed, varying, restless, upset, bored
Size of the pupils	☐		☐ strongly dilated, pointed
Pupils' reaction to light	☐		☐ slowed down, non-reacting
Nystagmus			☐ strong ☐ after following the object ☐ spinning induced
Other unusual findings		☐	☐ sweating, spasms, chills, dry mouth, running nose, tremor, watering or bloodshot eyes
Other observation:			

Samples* The skin was cleaned with ☐ water ☐ other. What? Blood sample/..... at ☐ 2 tubes according to the instructions ☐ other Urine sample/..... at under supervision ☐ yes ☐ no quality of urine ☐ normal ☐ unusual How? Urine test slip glucose ☐ no ☐ yes keto compounds ☐ no ☐ yes

Signature** Clarification of name and job position

Evaluation of the degree of the functional disorder (the total degree of errors)
1. Functional disorders ☐ were not observed ☐ were observed ☐ examinations were not carried out, because.....
2. The degree of the functional disorder ☐ is in the limits of normal variation ☐ deviates from the normal state and is at least ☐ mild ☐ of medium strength
3. To my knowledge these functional disorders/errors have been caused by ☐ drugs and/or ☐ medication and/or ☐ alcohol ☐ disease ☐ injury ☐ I can't evaluate

This I affirm by my honour and conscience

Date Signature

Clarification of name and job position.....

*Personal data and sampling time is written on the sample tubes.

**The signature of the person who took the samples, if not the same as the signature of this form.

Consent form

To the National Institute of Public Health

NAME OF INVESTIGATION: ROSITA – assessment of instant testing devices developed for identifying drugs

You are asked to participate in a mutual project of the European Union (EU) and the United States of North America (USA), where the instant testing devices developed for identifying drugs and their precision and usability are assessed. Pharmaceuticals and drugs are measured from the saliva sample and compared to the results of the other samples collected from You at this occasion. To participate you only have to sign this paper and give a saliva sample.

CONSENT TO PARTICIPATE IN THE STUDY

I am aware that when consenting to give saliva sample:

1. The sample will be used only with purpose for research.
2. The results are completely confidential.
3. My personal records are not combined with the results.
4. The device for collecting saliva is sterile.
5. **The results of the saliva test can not be used as evidence in legal proceedings.**
6. My participation in the saliva test is voluntary and I can refuse without implications. I can also call off the saliva test and my involvement in the saliva test at any time.

I have comprehended all of this and consent by signing to participate and agree to give a saliva sample for research purpose.

If any questions arise about the research project or your right as participant, you may contact following persons:

THANK YOU FOR YOUR CONTRIBUTION

The following definitions were used in the evaluation of the Drugwipe results:

True positive (TP): The case was considered true positive if Drugwipe result was positive and GC–MS confirmation result for oral fluid and/or whole blood (depending on the evaluation) was above the Strasbourg cut-off value for a given substance.

False positive (FP): The case was considered false positive if Drugwipe result was positive and GC–MS confirmation result for oral fluid and/or whole blood (depending on the evaluation) was below the Strasbourg cut-off value for a given substance.

False negative (FN): The case was considered false negative if Drugwipe result was negative and GC–MS confirmation result for oral fluid and/or whole blood (depending on the evaluation) was above the Strasbourg cut-off value for a given substance.

True negative (TN): The case was considered true negative if Drugwipe result was negative and GC–MS confirmation result for oral fluid and/or whole blood (depending on the evaluation) was below the Strasbourg cut-off value for a given substance.

Sensitivity= $TP/(TP + FN)$

Specificity= $TN/(TN + FP)$

Positive predictive value (PPV) = $TP/(TP+FP)$

Negative predictive value (NPV) = $TN/(TN+FN)$

Accuracy = $(TP + TN)/(TP+TN+FN+FP)$

Prevalence= $(TP+FN)/(\text{number of subjects})$

QUESTIONNAIRE FOR THE USERS OF THE DRUGWIPE ORAL FLUID TEST

Please circle the most suitable choice or fill in the blanks in this form. If extra space is needed, you may continue your answer on the reverse side of the paper.

ABOUT THE USER

1. Town and police district

2. Evaluate how many times you have used the following Drugwipe oral fluid tests:

Drugwipe 5 _____ times, of which the test didn't work for _____ times (no control line obtained)

Drugwipe benzodiazepine _____ times, of which the test didn't work for _____ times (no control line obtained)

COLLECTION OF THE ORAL FLUID SAMPLE

3. What kind of problems have you encountered during the collection of the oral fluid sample? Circle all the suitable options.

- 1 insufficient volume of oral fluid
- 2 subject's unwillingness to participate
- 3 refusal of the subject
- 4 reliability of the test questioned
- 5 aggressive behavior while testing
- 6 attempt of manipulation
- 7 working in a hygienic manner was difficult
- 8 other:

4. How did you solve the problems you encountered?

THE TEST CHARACTERISTICS

5. Please evaluate the characteristics of the test

1 – bad/weak

2 – slightly bad/weak

3 – mediocre

4 – good

5 - excellent

ease of use	1	2	3	4	5
ease of interpreting the test	1	2	3	4	5
credibility of the test	1	2	3	4	5
user instructions/training	1	2	3	4	5
size and appearance of the test	1	2	3	4	5

suitability for on-site testing 1 2 3 4 5

6. The good qualities and the weak points of the test at on-site testing. For example, does time of the day or season have an effect on performing the test?
7. Estimate the time required to carry out the test
_____minutes
8. Is the time required to carry out the test in your opinion
1 adequate
2 too long
9. Did you encounter any problems with the colouring of the test lines? For example, did it take longer than 5 minutes for a test line of a certain analyte to appear?
10. There is an optical reader device under development for the Drugwipe test. Do you think that this kind of device would be useful when interpreting the results?
1 yes
2 no
3 depends on the
situation:_____
11. In future I would prefer using
1 the Drugwipe oral fluid test
2 other / a better drug test
3 no drug test
12. Did you use the Intercept oral specimen collection device during the ROSITA2 project? How did you find the device/collection?
13. Other comments

THANK YOU FOR YOUR COOPERATION!

Appendix 6 Drugwipe device evaluations for each drug group

Table 1. Drugwipe device evaluation based on oral fluid GC-MS confirmation results.

	Amphetamine	Cannabis	Cocaine	Opiates	Benzodiazepines
TP	128	12	1	4	29
FP	1	11	1	6	6
FN	6	11	1	0	10
TN	13	114	145	138	32
Total	148	148	148	148	77
Sensitivity	95.5%	52.2%	50.0%	100.0%	74.4%
Specificity	92.9%	91.2%	99.3%	95.8%	84.2%
Accuracy	95.3%	85.1%	98.6%	95.9%	79.2%
PPV	99.2%	52.2%	50.0%	40.0%	82.9%
NPV	68.4%	91.2%	99.3%	100.0%	76.2%
Prevalence	90.5%	15.5%	1.4%	2.7%	50.6%

Table 2. Drugwipe device evaluation based on blood GC-MS confirmation results.

	Amphetamine	Cannabis	Cocaine	Opiates	Benzodiazepines
TP	216	28	2	7	50
FP	6	27	4	8	6
FN	5	13	2	1	25
TN	39	197	258	250	40
Total	266	265	266	266	121
Sensitivity	97.7%	68.3%	50.0%	87.5%	66.7%
Specificity	86.7%	87.9%	98.5%	96.9%	87.0%
Accuracy	95.9%	84.9%	97.7%	96.6%	74.4%
PPV	97.3%	50.9%	33.3%	46.7%	89.3%
NPV	88.6%	93.8%	99.2%	99.6%	61.5%
Prevalence	83.1%	15.5%	1.5%	3.0%	62.0%

Table 3. Drugwipe device evaluation based on oral fluid and blood GC-MS confirmation results.

	Amphetamine	Cannabis	Cocaine	Opiates	Benzodiazepines
TP	218	29	2	8	48
FP	5	26	4	7	9
FN	11	18	2	1	19
TN	33	193	259	251	46
Total	267	266	267	267	122
Sensitivity	95.2%	61.7%	50.0%	88.9%	71.6%
Specificity	86.8%	88.1%	98.5%	97.3%	83.6%
Accuracy	94.0%	83.5%	97.8%	97.0%	77.0%
PPV	97.8%	52.7%	33.3%	53.3%	84.2%
NPV	75.0%	91.5%	99.2%	99.6%	70.8%
Prevalence	85.8%	17.7%	1.5%	3.4%	54.9%

Chapter 6: Germany – Evaluation of on-site oral fluid test devices

M.R. Moeller, K. Hammer, H.-J. Maurer, R. Urban, J. Roehrich, O. Joesch, T. Jung

Abstract

In 2003 the European project ROSITA-2 started with the intention to evaluate the newest on site tests for the detection of drugs of abuse in oral fluid. For Germany the Institute of Legal Medicine in Homburg and the Saarland Police performed the first part of the study and the second part was performed with the Institute of Legal Medicine in Mainz and the Rhineland-Palatinate Police. During the field study 296 drivers agreed to perform an oral fluid on site test, 4 systems have been tested and 109 oral fluid samples have been analysed using GC/MS. The evaluated test systems were: the Oratect™ by Branam Medical Corp. was first tested in Saarland, but after the great number of test failures the evaluation has been stopped and an improved version has been tested in Rhineland-Palatinate after a few months. The evaluation of this new version was also cancelled, because the performance was significantly improved but still not sufficient. The Draeger DrugTest® (evaluated in Saarland) showed promising analytical and practical results but the production of the system was stopped at the end of 2005. The Rhineland-Palatinate Police also tested the Lifepoint®IMPACT® system which had a completely new way of sample collection, that can provide an exactly defined amount of oral fluid for the confirmation analysis. The evaluation has been stopped when the company closed its business in 2005. In 2005, a new system by Mavand, the OralStat® has been tested in Rhineland-Palatinate. The analytical data have to be improved since they do not meet the criteria for an acceptable test. However, the test system has some promising features. All systems have cut-off values for THC that are not low enough to meet the requirements needed in Germany. At present none of the evaluated test systems for oral fluid is acceptable for the police in Germany.

Introduction

The EU-project ROSITA – **R**oadside **T**esting **A**ssessment has been carried out between 1999 and 2000 in 8 European countries. The German part was performed by the Institute of Legal Medicine in Homburg and the Saarland Police [1]. Since ROSITA-1 the Saarland Police uses the Mahsan-Test for the detection of illicit drugs in urine. The use of on-site tests in urine has some negative side effects, e.g. if no restrooms are available at the testing site, female driver can not give a urine sample. Furthermore, since the positive detection of the listed drugs in blood is required in order to fulfil an administrative offence, urine testing can only be a hint for the recent consumption of cannabis, because the detection window in urine is behind the detection window in blood.

Police is seeking a test system which is easy to handle, quick and gives reliable results. One important point is the handling of human specimen. Police officers are not trained in handling human specimens like lab workers and they are often not willing to have close contact to human specimens. In order to raise the willingness of the officers to perform a test, a system is required which reduces the contact with the specimen to a minimum level or even better to avoid direct contact. The ideal system should also be quick (2 – 5 minutes), relatively insensitive to low or high temperatures and portable.

Since the great success of the first project, where mostly on site tests for urine have been investigated, a new project followed in 2003– ROSITA 2. The goal of this project was the evaluation of the newest oral fluid test systems with respect to the following aspects:

- the practical usability at roadside-testing and/or at the police stations for police officers

- the reliability of the tests based on the comparison of the on-site test results and the results of the confirmation analyses in oral fluid using GC/MS or LC/MS
- the correlation of on-site-test results and results of the blood analysis
- correlation of blood and oral fluid results for the determination of recent drug consumption, i.e. the definition of detection windows in oral fluid

In order to realize this intent the official partner in Germany, Prof. Dr. Manfred Moeller supervised the project in cooperation with the Institute of Legal Medicine in Homburg and the Saarland Police in the first part of the project and the Institute of Legal Medicine in Mainz and the Rhineland-Palatinate Police in the second part of the study. The field study was performed from December 2003 to August 2004 in Saarland and from July 2004 to November 2005 in Rhineland-Palatinate.

Methods

In 1998 a new law came into action in Germany. In contrast to the existing law which sanctions Driving under the Influence of Drugs (DUID) as a criminal offence (§ 316 of the penal code (StGB)) the new law created sanctions for driving under the influence of certain drugs that are listed in the appendix of the new law, as an administrative offence (§ 24a of the Road Traffic Law (StVG)). The listed drugs in the appendix are: amphetamine, MDMA, MDE, cannabis, cocaine, heroin and morphine. If one of these drugs is detected in blood (the target analytes for heroin and cocaine are morphine and benzoylecgonine, respectively and THC for cannabis) the new law is applicable. For the administrative offence following § 24a StVG only the evidence of recent consumption of one or more of the listed drugs is necessary. This evidence is given, if the presence of one of these drugs in blood is proven. This is a zero-tolerance law in its intention. However, cut-offs are defined for the different drugs/drug-metabolites: 1 ng/ml for THC, 10 ng/ml for morphine, 25 ng/ml for amphetamine, MDMA and MDE, 75 ng/ml for benzoylecgonine.

To constitute a criminal offence (§ 316 StGB) some additional criteria have to be fulfilled, e.g. the observation of signs of impairment within the area of vegetative symptoms (pupil width, pupil reaction), disturbances in motor control (walking, physical tests) or psychic disturbances (cognition, mood) must be present in order to prove driving inability. For the detection of those drivers who recently consumed illicit drugs (that are listed in the appendix of § 24a StVG) and do not show clear signs of impairment as described above, an on-site test is helpful.

The Saarland police and the Rhineland-Palatinate police usually collect the oral fluid samples during their routine work on the road; either stopping cars in moving traffic or at road blocks on special occasions, e.g. big music events or close to a disco on a weekend checkpoints have been set up. At such checkpoints, all passing cars can be checked, but not all drivers are subjects for closer inspections. The traffic is slowed down to walking speed, so that the police officers can have a look into the car. Usually younger drivers are stopped. The police officers ask routine questions and test the drivers for signs of impairment or signs for drug consumption (see Appendix 2: checklist). If they have reason to believe that the driver is under the influence of drugs or had used drugs recently, a urine on-site test (Mahsan DOA4) will be performed if possible. In positive cases, the drivers are asked if they are willing to perform an oral fluid on-site test. If one of the on-site tests is positive or clear signs of impairment are visible, a blood sample is taken at the police station.

The police officers were instructed to perform at least one test and collect an additional saliva sample for confirmation (via InterceptTM, except with Lifepoint[®]IMPACT[®] and OralStat[®] who had their own collection system for confirmation samples).

For the on-site tests special data sheets have been given to the police officers (see Appendix 1: Begleitbogen).

On-site Tests

In order to obtain a sufficient number of cases for the evaluation of the on-site tests the goal was to collect 125 tests for each device if possible. If it was clear that a test was not satisfying, because e.g. the failure rate was too high, the field testing was stopped and if possible redone with an improved on-site test. The first part of the study was performed in Saarland. It was drawn by lot which country would have to evaluate which test.

Germany started the project with the OratectTM Multiple Drug Screen Oral Fluid Test by Branan Medical Corporation (Irvine, USA). The OratectTM is a “one-step lateral flow immunoassay device for the qualitative detection of THC (TH), Methamphetamines (ME), Cocaine (CO), Amphetamines (AM), Opiates (OP), Phencyclidine (PC), and their metabolites in human oral fluid”. The test is small and can be carried around without the need for a reading unit. For the collection of oral fluid an exactly described procedure has to be followed and the collection time should be 5 minutes. The results should be read within 8 to 10 minutes after the collection. The total testing time should be 13 to 15 minutes.

The second test was the Draeger DrugTest[®] (Luebeck, Germany) which is an instrument-based test system that requires a reading unit which can be located at the police station or in a police car. The test cassette and the sample collector are separated and have to be plugged together after the sampling. The sample collection does not follow a specific procedure and the status can be checked during the procedure. After the collection, the results should be available within 10 minutes. The detectable substances are: THC, amphetamine, methamphetamine/MDMA, opiates and cocaine.

For the lab confirmation an additional oral fluid sample was collected using the InterceptTM by Orasure Technologies, Inc. (USA). This sample collector consists of a salty collection pad and a tube with a buffer solution for the storage of the oral fluid sample.

The Rhineland-Palatinate Police tested an improved version of the Branan OratectTM and additionally the new instrumented test system Lifepoint[®] IMPACT[®] which does not collect the oral fluid sample with a cotton pad but by using a suction pump, similar to a dentists pump. The sampling procedure should take 5 minutes and the analysis time another 5 minutes which gives a total testing time of 10 minutes. This test system can be performed at a police station but also in a police car if a table or even surface is available.

A new test system has been evaluated in Rhineland-Palatinate. The Oralstat[®] by Mavand Solutions GmbH (Moessingen, Germany) is an immunological on-site test with an optional electronic reader. The system includes a collection stick, similar to the stick available from Draeger, which makes it possible to check the status of the collection procedure, buffer, a test cassette and a reading unit. The reaction time of the immunological test takes 8 minutes and the reading time is 2 – 4 minutes. The detectable substances are: amphetamines, benzodiazepines, cocaine, methamphetamine/MDMA, opiates, phencyclidine and THC.

The police in Rhineland-Palatinate also collected additional Oral fluid samples using the InterceptTM collector when testing the OratectTM. With the Lifepoint[®] IMPACT[®] system and the Mavand Oralstat system it was possible to collect a second sample for the confirmation analysis.

Sample collection

OratectTM Multiple Drug Screen Oral Fluid Test

The collection pad of the Oratect had to be wiped on the left and the right side of the tongue for 5 seconds each and after that placed under the tongue pressing lightly on the bottom of the mouth until the flow started (this should have happened within 5 minutes). After recapping the test, the results should have been read within 8 to 10 minutes.



Fig. 1: Oratect™ test device – first version tested in Saarland

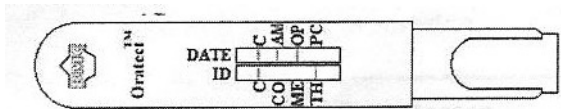


Fig. 2: Oratect™ test device – second version tested in Rhineland-Palatinate

Draeger DrugTest®

The collection stick of the Draeger test system consists of a small pressed sponge which expands when it comes in contact with saliva. The stick has to be moved around in the mouth of the subject until enough oral fluid has been collected. This could be checked during the procedure by visual control of the sponge expansion. If not enough fluid had been collected the procedure could be continued and the sponge expansion rechecked. If enough fluid was collected, the stick was put into the test cassette which was placed into the reading unit.



Fig. 3: Draeger DrugTest®

Intercept™

For the confirmation analysis an oral fluid sample collector was used. The Intercept™ is a collection stick with a salty cotton pad which had to be wiped inside the cheeks on both sides and then placed there for 2 to 5 minutes. The pad was stored in the tube which contained a buffer solution for conservation.



Fig. 4: Intercept™

Lifepoint® IMPACT®

After inserting the test module, the test person had to put the mouthpiece into his oral cavity and the aspiration process could be started. This should take about 1 ½ up to 5 minutes. After that the analysis starts and takes about 5 minutes.



Fig. 5: Lifepoint® IMPACT®

OralStat®

The collection stick consists of 3 pressed sponges, which expand when the collector stick has to be placed inside the cheeks on every side for about 30 seconds until the sponge is expanded. After the sample collection 12 – 15 drops of buffer have to be added to the sponge. The test stripe with the antibodies has to be inserted in the testing chamber which contains two buffer reservoirs and the chamber has to be moved in circles on a plane surface for 3 seconds. The sponge is inserted until a first click is heard and after 5 seconds the stick has to be turned and pushed down. After another 5 seconds the whole cassette has to be turned in circles for 3 seconds. The test stripe has to be pushed down and the reaction time starts. The result can be read visually and with the help of the reading unit.



Fig. 6: OralStat®

Table 1: Cut-off values of the on site tests [ng/ml] for oral fluid

	Amph.	Methamph./MDMA	Coc.	Opi	THC
Oratect™	50	50	20	20	100
DrugTest®	10	10	5	5	20
Lifepoint®	50	50	20	40	15
OralStat®	25	25	12	20	25

Lab Analyses

The blood samples collected during the first part of the project were analysed within the routine lab procedure at the Institute of Legal Medicine in Homburg/Saarland. The samples are screened with enzyme-immunoassays from BioRad (Munich, Germany) for the following substances: methadone, amphetamines, methamphetamines, opiates, cocaine and cannabis or for a selected group of substances if there is an indication given by on-site tests or by urine analysis. Positive samples are confirmed by GC/MS analysis. After solid phase extraction PFP derivatisation or methylation is performed [2]. The oral fluid samples have been analysed at the Institute of Legal Medicine in Mainz.

The lab procedure at the Institute of Legal Medicine in Mainz is comparable to the procedure in Homburg. If urine samples are available, they will be screened with immunoassays from Microgenics for amphetamines, cannabis, cocaine, opiates, benzodiazepines and methadone. If only blood samples are available they will be screened with enzyme-immunoassays from MAHSAN for the same substances or for a selected group of substances if there is an indication given by on-site tests or by urine analysis. Confirmation analysis of positive screening results is performed with GC/MS. The oral fluid analyses are performed with the same methods as used for blood with validated methods [3].

Statistical analysis has been performed after the following definitions:

- **Sensitivity:**
$$\frac{\text{correct positive}}{\text{correct positive} + \text{false negative}}$$
- **Specifity:**
$$\frac{\text{correct negative}}{\text{correct negative} + \text{false positive}}$$
- **Accuracy:**
$$\frac{\text{correct positive} + \text{correct negative}}{\text{correct positive} + \text{false positive} + \text{correct negative} + \text{false negative}}$$
- **Positive Predictive Value (PPV):**
$$\frac{\text{correct positive}}{\text{correct positive} + \text{false positive}}$$
- **Negative Predictive Value (NPV):**
$$\frac{\text{correct negative}}{\text{correct negative} + \text{false negative}}$$

Results

In general

During the whole project 296 tests in oral fluid have been performed; 147 in Saarland and 149 in Rhineland-Palatinate. In Saarland 63 tests using the Oratect™ (first version) and 134 tests using the DrugTest® have been performed.

Table 2: number of test failures with the Oratect™ (first version) in Saarland

Performance	number of tests	percentage
working	9	14,3
half working	4	6,3
not working	50	79,4
total	63	100

Table 3: number of test failures with the DrugTest® in Saarland

Performance	number of tests	percentage
working	120	89,6
not working	14	10,4
total	134	100

In Rhineland-Palatinate 149 tests have been performed. 53 tests were performed using the improved version (version 2) of the Oratect™, 44 using the Lifepoint® test system and 52 tests with the OralStat®.

Table 4: number of test failures with the Oratect™ (improved 2nd version) in Rhineland-Palatinate

Performance	number of tests	percentage
working	33	62,3
not working	20	37,7
total	53	100

Table 5: number of test failures with the Lifepoint® IMPACT® in Rhineland-Palatinate

Performance	number of tests	percentage
working	30	68,2
not working	14	31,8
total	44	100

Table 6: number of test failures with OralStat® in Rhineland-Palatinate

Performance	number of tests	percentage
working	49	94,2
not working	3	5,8
total	52	100

Testing related aspects

The collection of the oral fluid samples was for no system “unacceptable” for the police officers, which means that the handling was hygienic and no direct contact of the police officers with the sample was necessary. The different test systems had very different difficulties in sample collection. The first version of the Oratect™ had great difficulties in sample collection. The amount of oral fluid collected for a valid test was mostly not enough and the procedure too complicated so in most cases there was no testing possible (79,4%). The sample collection time was much longer than 5 minutes. It seemed that the test persons could outsmart the test by not following the instructions and only 20,6% of the tests showed any result, so testing were stopped after 63 cases. Branan Medical provided an improved version of the test after a few months and the new test had a shorter collection pad, so less oral fluid was necessary. The number of tests that collected enough oral fluid (62,3%) was much higher but still not sufficient. If the tests were working the results could easily read without any difficulties.

The sample collection with the collection sticks of the Draeger DrugTest® was easy, especially because the status of the procedure could easily checked by looking at the expansion grade of the sponge, if not sufficient, the collection could be continued. Such an interruption of the procedure was particularly prohibited for the Oratect™. None of the invalid tests was due to difficulties in sample collection. The reading of the results with Draeger DrugTest® was in 11,8% of the cases not possible because no reading unit was available or the reading unit had a software problem and could not read the test cassette. The software was updated which should have limited that problem. The handling of this test system was easy but an additional instrumentation was necessary. That means that this test was not completely portable.

The Lifepoint® IMPACT® presented a new way of sample collection, comparable to a saliva aspiration pump at the dentist. This seemed to provide an exact amount of oral fluid for the testing procedure. However, in many cases the sample collection was not sufficient because the instrument stopped the sampling automatically after a preset time, which could not be

extended, even if not enough oral fluid was collected. The testing was cancelled when the company Lifepoint has closed its business.

Since the sample collection with the OralStat[®] works in the same way as the DrugTest[®] difficulties were not expected, but the collection stick of the OralStat[®] lost one of its collection sponges in some cases.

In contrast to the Draeger system the Mavand test could also be read without the reading unit. The scanning of the test strip was sometimes difficult because the system showed wrong results if the test strip was not in the exact position. This made the handling difficult for the officers.

Analytical results

Oratect[™] in Saarland (first version)

Due to the low number of working tests the test has not been analytically evaluated.

Oratect[™] in Rhineland-Palatinate (improved version)

The working tests were all negative, so no analytical evaluation has been performed.

Draeger DrugTest[®]

Table 7: DrugTest[®] test results compared to blood values in 108 cases

Substance	THC	Amphet- amine	Methamphetamine/ MDMA	Opiates	Cocaine
correct positive	46	13	10	4	8
false positive	4	6	1	0	6
correct negative	47	76	93	101	92
false negative	10	13	4	3	2
Sensitivity	82,1 %	50,0 %	71,4 %	57,1 %	80,0 %
Specificity	92,2 %	92,6 %	98,9 %	100,0 %	93,8 %
Accuracy	86,1 %	82,4 %	95,4 %	97,2 %	92,6 %
PPV	92,0 %	68,4 %	90,9 %	100,0 %	57,1 %
NPV	82,5 %	85,4 %	95,9 %	97,1 %	97,9 %
PPV = positive predictive value; NPV = negative predictive value					

Table 8: DrugTest[®] test results compared to oral fluid values in 65 cases

Substance	THC	Amphet- amine	Methamphetamine/ MDMA	Opiates	Cocaine
correct positive	29	6	3	1	4
false positive	0	6	4	0	3
correct negative	32	52	58	62	55
false negative	4	1	0	2	3
Sensitivity	87,9 %	85,7 %	100,0 %	33,3 %	57,1 %
Specificity	100,0 %	89,7 %	93,6 %	100,0 %	94,8 %
Accuracy	93,9 %	89,2 %	93,9 %	96,9 %	90,8 %
PPV	100,0 %	50,0 %	42,9 %	100,0 %	57,1 %
NPV	88,9 %	98,1 %	100,0 %	96,9 %	94,8 %
PPV = positive predictive value; NPV = negative predictive value					

Lifepoint[®] IMPACT[®]

Since the company closed business during the test phase, the results have not been evaluated.

OralStat[®]

Table 9: OralStat[®] test results compared to blood values in 35 cases

Substance	THC	Methamphetamine/ Amphetamine	Opiates	Cocaine
correct positive	2	13	0	2
false positive	3	1	1	2
correct negative	17	18	34	29
false negative	13	3	0	2
Sensitivity	13,3 %	81,3 %	- %	50,0 %
Specificity	85,0 %	94,7 %	97,1 %	93,5 %
Accuracy	54,3 %	88,6 %	97,1 %	88,6 %
PPV	40,0 %	37,1 %	0,0 %	50,0 %
NPV	56,7 %	85,7 %	0,0 %	93,5 %
PPV = positive predictive value; NPV = negative predictive value				

Table 10: OralStat[®] test results compared to oral fluid values in 44 cases

Substance	THC	Methamphetamine/ Amphetamine	Opiates	Cocaine
correct positive	8	19	0	2
false positive	1	1	2	2
correct negative	16	19	42	38
false negative	19	5	0	2
Sensitivity	29,6 %	79,2 %	- %	50,0 %
Specificity	94,1 %	95,0 %	95,5 %	95,0 %
Accuracy	54,5 %	86,4 %	95,5 %	90,9 %
PPV	88,9 %	95,0 %	0,0 %	50,0 %
NPV	45,7 %	79,2 %	0,0 %	50,0 %
PPV = positive predictive value; NPV = negative predictive value				

Discussion and Conclusions

For all tested on-site systems cannabis was the most critical substance but in Germany cannabis is the most important substance to detect since 60 – 80% of all DUID cases are cannabis cases. However, sensitivity for THC has to be improved for roadside testing in Germany.

The Oratect[™] by Branan Medical would be an ideal test system, because it is very small and portable; every police officer could take some tests with him during his routine work. No additional equipment is needed. But the sample collection is too complicated and can be outsmarted by the tested persons and takes too much time. The tests is probably very suitable for other testing circumstances, e.g. jail, rehabilitation centre or any other case where the test person is really willing to co-operate (which is not the case, if a driver is stopped and has to fear to be tested positive). Additionally it seems that both test versions (version 1 tested in Saarland and improved version 2, tested in Rhineland-Palatinate) are not sufficient in sensitivity for THC as far as any conclusion can be drawn from the few working tests.

The Draeger DrugTest[®] was very well accepted by police officers and tested persons, even though a reading unit was necessary. The sample collection was very easy, hygienic and the most important point was that the collection status could be checked during the procedure. The analytical results show that there are difficulties in the detection of amphetamines and designer drugs with respect to false positive/negative results [4]. The stated cut-off levels for THC (Tab. 1) are too high in all tested systems, since such concentrations can only be found within 1 or 2 hours after the consumption [5]. Recent controlled laboratory experiments showed that even with high THC doses (approx. 35 mg /subject) after two hours mean THC-values are below 10 ng/ml. However, significant signs of impairment can be measured in tracking experiments down to 2 ng/ml after 5 -6-hours [6]. The Draeger DrugTest[®] was the best system concerning the acceptance by police officers and drivers and the analytical data. In 2005 the production of the Draeger DrugTest has been stopped and this system won't be available any longer.

The evaluation of the Lifepoint[®]IMPACT[®] system was stopped after the company closed its business. Even though the sample collection had some difficulties, this collection system seemed to be a good idea, especially because it was possible to collect a defined amount of oral fluid, which makes a quantitative analysis of the oral fluid possible. No other system had the possibility to collect an exactly defined amount of oral fluid for the confirmation analysis. The OralStat[®] by Mavand was a new test system that has some promising features. Comparable to the Draeger DrugTest[®] it has a similar collection system, which can also be checked during the sampling procedure. But in this case the used sponges do not stick to the collection stick properly; in some cases one of the 3 sponges “fell off” the stick even in cases of proper application. The analytical part has also to be improved. The cut-off for THC is too high and the sensitivity is not sufficient. The time needed for a test performed with the OralStat[®] is much too high compared to urine tests, which reduces the acceptance of the test among the police officers.

All systems are far away from the originally defined target of 90% levels for the parameters: sensitivity, specificity, accuracy, PPV and NPV. However, these requirements are indispensable for a system which is acceptable for police officers in road side tests. With lower defined targets for the 5 parameters, saliva test systems will not be accepted by police officers in Germany.

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An die
Universität des Saarlandes
Institut für Rechtsmedizin, Gebäude 42
Homburg

FAX
An die
Landespolizeidirektion
SB 13 Fax: 0681/9622036

**Drogenvortest – Begleitbogen
zum EU-Forschungsprojekt ROSITA 2
„Korrelation von Blut / Speichel bei Drogeneinfluß“**

Bitte ankreuzen und ausfüllen

VPI	☐		PB	☐		KPI	☐	
§ 24a StVG ☐ § 316 StGB ☐ Sonstiges Delikt: ☐ Aktenzeichen:.....								
Tatzeit:	Nr. des roten Klebezettels:		Uhrzeit Entnahme:		Umgebungstemperatur			
Datum	Blut		Blut		(wenn möglich)			
Uhrzeit	Urin		Urin					

Eingesetzte Vortestmöglichkeiten: (bitte ankreuzen)

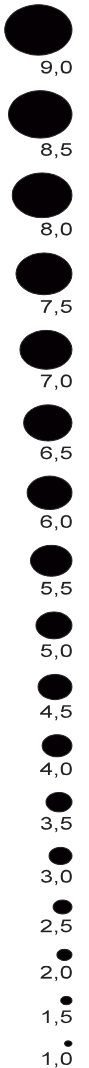
Forschungsprojekt „ROSITA 2“		+	-	Erste Anzeige nach ... Minuten ab Teststart	Testzeit (Uhrzeit)		Bemerkungen (z.B ungewöhnlich lange Reaktionszeit bei niedrigen Temperaturen)
					Beginn	Ende	
Dräger	THC						
	OPI						
	COC						
	Amp						
	Meth						
Oratect	CO						
	ME						
	TH						
	AM						
	OP						
	PC						
Intercept							
Eingesetzte Testverfahren LPD							
MAHSAN	THC						
	Amphetamin						
	Opiate						
	Cocain						
	Meth						
Drugwipe	Amphetamin						
ONTRAK TESTSTIK 3	THC						
	Cocain						
	Morphin						
				93			
Sachbearbeiter							

Appendix 2: checklist of the Saarland Police

Police observations on reduction in driving fitness

Annex to report on		
Surname:	First name:	Date:
Incident:		Blood test no:
Observations on driving style, weather and road conditions		
Driving style: ☐ no own observations ☐ safe ☐ unsafe ☐ swerving about - deviation from straight line by up to m - number of swerves - observed over a distance of m ☐ unsuitable speed ☐ right of way ignored ☐ attracted attention in other way	Vehicle operation: ☐ stalled engine ☐ unsure gear changes ☐ engine roaring ☐ other	Vehicle faults: ☐ no ☐ yes, describe
Road condition: ☐ good ☐ bad ☐ work site ☐ well lit ☐ poorly lit ☐ dry ☐ wet	Weather conditions: ☐ rain ☐ ice / snow ☐ strong wind / storm ☐ fog ☐ daylight ☐ dusk ☐ dark	
Observations when stopped or encountered		
Reaction: ☐ normal ☐ delayed ☐ extremely slow	Unusual physical signs: ☐ none ☐ sweating ☐ shaking ☐ agitated ☐ vomiting	Appearance: ☐ clean and tidy ☐ unkempt ☐ neglected
Command of German language: ☐ yes ☐ no ☐ limited	Speech: ☐ clear ☐ stuttering ☐ slurred ☐ mumbling	Response / orientation: ☐ sleepy ☐ easy to wake ☐ in deep sleep / unconscious ☐ orientated ☐ confused
Mood / behaviour: ☐ quiet, in control ☐ excited ☐ strangely cheerful ☐ impassive ☐ doesn't keep distance ☐ provocative ☐ aggressive ☐ tearful	Getting out of the vehicle: ☐ normal ☐ unbalanced ☐ has to hold onto vehicle	Walk: ☐ steady ☐ dragging ☐ unsteady ☐ staggering
Smell of alcohol: ☐ yes ☐ no		
Alcohol test ☐ yes, at a.m./p.m.°/oo ☐ no ☐ refused ☐ cannot be carried out		
Eyes: ☐ normal ☐ red conjunctiva ☐ watery / shiny ☐ agitated	Pupils: right left ☐ approx. ...mm approx. ...mm ☐ immediate reaction to light ☐ slow reaction to light	Light conditions at place of examination: ☐ daylight ☐ dusk ☐ night / street lighting ☐ night / interior lighting
Other observations (all powders, tablets etc. found, other peculiarities in the car, on the person; continue on reverse side if necessary):		
Behaviour during official interview: (duration: from : till :) ☐ stayed the same ☐ increasingly strange ☐ became more normal		

mm



Observed by: _____
Name

Signature

V06-09-96

CHAPTER 7: EVALUATION OF DIFFERENT ONSITE DRUG TESTING DEVICES. THE NORWEGIAN PARTICIPATION IN THE EU-PROJECT ROSITA-II

Asbjørg S. Christophersen*, Åse Ripel*, Inge Frydenlund**

*Division of Forensic Toxicology and Drug Abuse, Norwegian Institute of Public Health
P.O. Box 4404 Nydalen, 0403 Oslo Norway

* * Oslo Police District, Operational Traffic Division, P.O. Box 8101 Dep, 0032 Oslo

Abstract

Four different onsite devices for drug screening in oral fluid (Oratect^R, DrugTest^R, Drugwipe^R, Oraline^R) have been evaluated in Norway as a part of the EU-project ROSITA. The evaluation was performed in collaboration with representatives from the police, testing suspected drugged drivers, drivers stopped by random control and drug addicts. All subjects participated on voluntary basis. The following characteristics of the devices were evaluated by the police: user-friendliness, suitability for roadside testing, time use from sampling to final results, reading of results, other characteristics important for the police during traffic control. Blood samples and/or oral fluid were collected simultaneously with the onsite testing. The oral fluid was analysed by LC/MS/MS for amphetamines, ecstasy, opiates, cannabis, cocaine and a number of benzodiazepines and their metabolites, evaluated as positive or negative according to standard cut-off limits decided by the project group. The blood samples were analysed at the Norwegian Institute of Public Health, Division of Forensic Toxicology and Drug Abuse (DFTDA) by the standard routine program used for drugged driving cases: Immunological and LC/MS–screening, followed by GC/MS or LC/MS quantification. The results from onsite screening using the devices were compared with the corresponding oral fluid and/or blood samples analyses.

Results: The different types of device had both positive and negative characteristics with regard to user-friendliness. However, too many invalid results were obtained. Comparison with results from blood and/or oral fluid analyses showed several deviations, mainly for tetrahydrocannabinol (THC) and benzodiazepines (BZDs) where improved sensitivity is necessary. Only one of the devices included BZDs, which are some of the most frequently detected compounds among drugged drivers in Norway. Benzodiazepines are of considerable interest in Norway for onsite drug testing using oral fluid, both for roadside screening of suspected drugged drivers and other cases. However, improved user-friendliness and increased sensitivity for some important drugs are necessary, before the authorities can accept the equipment to be used by the police.

Introduction

Drugs and driving in Norway

The Norwegian Road Traffic Act prohibits driving under the influence of alcohol (limit 0.02%), illicit drugs and psychoactive medicines. No legal limits have been established for drugs other than alcohol and each case of suspected drugged driving has to be evaluated for the court with regard to possible impairment. This evaluation is based on results from a clinical examination of the suspect performed by a physician at the time of blood sampling, interview and other relevant information from the police and results from blood sample

analyses. In most cases an expert witness statement is prepared for the court (1). However, legal limits for some of the most commonly detected drugs among apprehended drivers are discussed, recently also proposed by an official committee appointed by the Ministry of Transport (2).

The main reasons for apprehension of suspected drugged drivers are accidents, reckless or dangerous driving, or reports to the police by other road users. Relatively few drivers are arrested in general roadside controls. For many years, a high detection rate of driving under the influence of drugs other than alcohol has been recorded in Norway, variable from 4 – 5000 cases each year (4,5 million inhabitants) (3). Reduced resources in the police districts are one of the main reasons for reduced apprehension rate as each district has to pay for the cases. Previously, the expenses were covered by the Ministry of Justice.

According to the instruction for control of driving under the influence, a Norwegian police officer can stop a driver for alcohol screening using a breathalyzer, without any suspicion of driving under the influence. Screening for alcohol is also performed in connection with other type of traffic control, giving the police the opportunity to evaluate possible drug influence. The Norwegian police has no equipment to perform roadside drug testing. However, a large number of police officers involved in traffic control have been trained to perform investigation based on a “Drug Recognition Expert Program”. Detection of syringes, pills or equipment connected to drug use, will strengthen their suspicion towards drugs other than alcohol. Since many years, the Norwegian police have frequently directed their focus towards other drugs, and therefore gained valuable experience in the selection of drugged drivers. In spite of the unavailability of onsite equipment for primary drug screening, one or more illegal or psychoactive medicinal drugs are detected in approximately 80% of the cases where such substances have been suspected. For some cases of no drug detection, an accident may be the primary cause for apprehension, as alcohol and drugs analyses are important for evaluation of the responsibility of the incident.

The most commonly detected substances among drivers suspected to be influenced by other drugs than alcohol are THC, amphetamines, BZDs, (most frequently diazepam, flunitrazepam, clonazepam, nitrazepam, alprazolam), opiates (morphine, codeine, 6-monoacetylmorphine (6-MAM)). The hypnotic zopiclone and muscle relaxants carisprodol and meprobamate are also frequently detected. Cocaine and ecstasy are more unusual among apprehended drivers. (3).

In spite of the high detection rate, the Norwegian police have for a long time expressed a need for equipment to be used primarily at the roadside for evaluation of possible recent drug use, before the decision to bring the drivers to the police station and further call a physician. In some cases, the suspected drivers may be released, due to missing signs of impairment or other events to support the primary suspicion of drugs influence. The suspicion of medicinal drugs (e.g. BZDs) may be difficult to confirm. Thus, it is important that future on-site devices cover the most frequently detected drugs important for traffic safety. Thus, the Norwegian police have been positive to evaluate onsite devices for drug testing in oral-fluid, to give their opinion of user-friendliness, time use, including and suitable for roadside use.

Methods

Formal application - ethical committee

An application was sent to The Regional Ethical Committee, including description of the project, the sampling procedure and oral fluid analyses. They had no objection to the project assuming that participation from all test persons was voluntary, and that oral fluid samples collected for confirmation analyses were destroyed after final analyses.

Formal application - police collaboration.

A formal application was sent to the Norwegian Police Directorate, asking for participation from the police districts. Training, information meetings, travel expenses for the police should be covered by the project. Extra work for the police officers involved in the project had to be covered by the police districts.

The Police Directorate selected four different districts that received a formal request for participation, including description of the project, a list of tasks for the police, and to select a contact person for collaboration directly with DFTDA. The four selected districts were: Oslo, Asker and Bærum, Søndre Buskerud (both in the area close to Oslo) and Rogaland (the South-West coast of Norway). In addition, the Mobile Force Traffic Police was asked to participate during some roadside controls. All districts accepted to participate.

Requirements from the police

The police requirements for onsite devices were i) user-friendliness ii) simple test procedure iii) results within 5 minutes or less – preferably shorter than 2 minutes (for larger roadside controls, the results should be available within the time-span required to control the driving licence and vehicle registration papers iv) easy to read the results – preferentially without reader v) covering important medicinal drugs (e.g. BZDs) vi) easy to use onsite at the road vii) useable at different weather conditions – temperature – from $> + 20^{\circ}\text{C}$ to $-$ below 10°C .

Selection of test subjects and information.

The devices should primarily be used to test suspected drugged drivers where blood (and urine) samples should be collected. Based on agreement with the Police Directorate, the test subjects could also be selected among drivers stopped by the police during random roadside control. In addition, to get sufficient number of drug positive samples, some drug addicts from the streets were also asked to participate. A minor number of tests were collected from employees at DFTDA connected to ongoing projects (medicinal drugs). Females were represented in about 10 – 15 % of the test subjects. All age groups were represented; however, the majority was below forty years old.

All subjects participated on voluntary basis. Information from the randomly selected drivers and the group of drug addicts, were anonymised. The test subjects received information of the project and were informed that they could refuse to participate, that results would have no legal purpose for the individual subjects and no legal sanction could be taken based on results from the devices or oral fluid confirmation analyses.

Places for testing

The Norwegian police have no special cars with facilities for drug testing and other related investigation. Motorbikes are sometimes used during traffic controls. All tests on suspected drugged drivers were therefore performed at the police station in connection with oral fluid, blood (and urine) sampling and clinical examination. All samples together with the protocol including the cases and the clinical tests of impairment were sent to DFTDA for analyses. Testing of drivers stopped by roadside controls and drug addicts, were all performed outdoors. Oral fluid for confirmation analyses was collected simultaneously. Thus, the time between onsite testing, oral fluid and blood sample collection was insignificant.

Information meetings - training of police officers

The first part of the project included information meetings with representatives from the different participating police districts. Trainings were organised at each police district, at least two times to cover the different onsite devices and the device used for oral fluid collection to be used for confirmation. Representatives from the project group at DFTDA performed training, in some cases in collaboration with a representative from the producer (Dräger). Several police officers from each district received training. Instructions how to use the devices and oral fluid collection device, were available from the producers, further modified

and translated by the project group at DFTDA. Posters with instructions were prepared for all police districts to display at the wall in connection with the equipment and testing place. A protocol was worked out for the police, including information of the project, instructions for the testing process, time and place for testing, onsite test results and police officer performing the test. Subject who refused and the number of invalid tests should be recorded (Appendix 1).

Testing period

The test period started in September 2004 and lasted to December 2005. However, no testing during the period from February 2005 until October 2005 was performed, due to waiting for new equipment.

Equipment - devices evaluated

The following onsite devices were evaluated:

DrugTest^R from Dräger (Lübeck, Germany) including an electronic reader (DrugTest^R Analyzer), covering amphetamine, methamphetamine, cannabinoids, opiates and cocaine.

Oratec^R from Branan Medical Corporation (Irvine, CA), covering amphetamine, methamphetamine, THC, opiates, cocaine and PCP.

Drugwipe^R (Securetec Detektion-Systeme AG, Brunthal, Germany) covering amphetamines, /methamphetamine/ecstasy, cannabis, opiates and cocaine. A separate device covering BZDs is available.

Oraline^R from Sun Biomedical Laboratories (Blackwood, NJ) covering methamphetamine, opiates, THC, cocaine.

Intercept^R devices from OraSure Technologies, (Bethlehem, PA) was used for oral fluid collection.

Reference biological materials

Results from on-site devices were compared with results from blood drug analyses (if available). In addition, results from specific oral fluid analyses, if available, were also used for comparison.

Handling of oral fluid samples - analytical methods

The Intercept^R devices were weighted for volume control of the collected oral fluid. The sample tubes were centrifuged and the oral fluid – mixture transferred to a new tube, stored at -4°C before analysis (normally within 1-2 weeks). No immunological method was used for primary screening. All samples were directly analysed using liquid chromatography/ mass spectrometry (LC/MS/MS) after liquid/liquid extraction followed by evaporation (4). Positive ionization was performed in the multiple reaction mode (MRM) for detection of the following compounds recommended by the ROSITA project group and also some other compounds frequently detected among drugged drivers in Norway: Amphetamine, methamphetamine, MDMA, MDA, MDEA, cocaine, benzoylecgonine, morphine, 6-MAM, codeine, THC, diazepam, nordiazepam, temazepam, oxazepam, alprazolam, flunitrazepam, 7-aminoflunitrazepam, nitrazepam, 7-aminonitrazepam, bromazepam, lorazepam, clonazepam, zopiclone, zolpidem, carisprodol¹, meprobamate¹ and buprenorphine¹ (4). The cut-off limits used were in accordance with recommendation from ROSITA project. The compounds included in the method are listed in table 1, including their cut-off limits. Information of additional included compounds is included in the table.

Table 1

Compounds and cut-off limits included in the analytical program used for blood and oral fluid analyses. When no comments, cut-off limits recommended by the ROSITA project are used.

Compound	Cut-off ng/ml Blood	Cut-off ng/ml Oral fluid	Comments
Amphetamines	20	25	
Metamphetamine	20	25	
MDMA	30	25	Higher blood cut-off value
MDA	30	25	“
MDEA	-	25	Not included in the standard blood method – not detected in Norway
THC	1	2	Standard cut-off in standard blood method: 0,3 ng/ml. ROSITA-samples:1 ng/ml:
Cocaine	30	8	Higher blood cut-off value
Benzoyllecgonine	30	8	Higher blood cut-off value
11-OH-THC	-		Not included in blood
THCCOOH	-		“
Morphine	10	20	
Codeine	10	20	
6-MAM	10	10	Not included in the ROSITA recommendation
Diazepam	50	5	
Nordiazepam	50	5	
3-OH-diazepam	–	5	Not registered in Norway
Oxazepam	0,5	5	Frequently detected in Norway
Flunitrazepam	1,5	2	Not included in ROSITA – frequently detected in Norway
7-aminoflunitrazepam	55	2	Higher blood cut-off value
Clonazepam	5	5	
7-aminoclonazepam	28	5	Metabolite not included in ROSITA
Nitrazepam	7	2	Not included in ROSITA – frequently detected in Norway
7-aminonitrazepam	10	5	”
Alprazolam	5	5	Not included in ROSITA – frequently detected in Norway
Lorazepam	-	5	Not in Norway
Bromazepam		5	Not registered in Norway – not detected
Zopiclone	20	10	Higher blood cut-off value
Zolpidem	15	10	Higher blood cut-off value

Standard routine for analyses of drugs in samples from apprehended drivers.

Blood samples (and urine in most cases) used for comparison in the ROSITA project, were handled according to the standard analytical program used for samples from apprehended drivers. An immunological method (EMIT) is used for the screening of amphetamines, ecstasy, opiates, cannabinoids and cocaine (5). Screening for psychoactive medicinal drugs is performed by LC/MS covering the following compounds: Diazepam, nordiazepam, nitrazepam, flunitrazepam, oxazepam, alprazolam, fenazepam¹, zopiclone, zolpidem.

methadone¹, carisprodol¹, meprobamate¹, dextropropoxyphene¹, phenobarbital¹, phenytoin¹, Urine samples are screened by EMIT, covering amphetamines, ecstasy, cannabis, opiates, cocaine and BZDs. Positive blood samples after screening are confirmed and quantified using GC/MS (amphetamines, ecstasy, THC, opiates, cocaine/benzoylecgonine) (6,7) and LC/MS (medicinal drugs) (8). Urine samples are confirmed after positive screening of opiates for the detecting of 6-MAM. All results are calculated according to calibrators made in whole blood. Program for independent control samples are included in all analyses. DFTDA regularly participates in several external quality control programs covering most of the compounds. The cut-off limits used for comparison of results from samples included in the ROSITA project were mostly in accordance with the recommendation from the project, except for some BZDs. The compounds included in the analytical program for drug analyses and their cut-off limits are listed in table 1.

Results and discussion

Testing of devices by the police

From the start of the project, the police were interested and positively engaged to evaluate the devices. Since long time, devices to be used at the roadside for pre-screening of suspected drugged drivers similar to alcometer, had been a prioritised desire. Based on information from the Ministry of Justice, such equipment should be available within short time. It was therefore important to test available equipment to find the most appropriate equipment for roadside use and to compare the positive or negative results with the corresponding blood sample analyses. During the evaluation of the first selected devices, several unexpected problems occurred. For one of the devices (Oratect^R), most of the results were invalid, which was discovered during training. A new batch of the device received from the producer, did not solve the problem. The other device that was tested (Dräger), was rather complicated to use with many steps before the results were available after at least 16 minutes. These unexpected problems resulted in a loss of motivation for several police officers. The available equipment was not what they needed. These primary problems resulted in few test for comparison with blood sample analyses.

Evaluation of the different devices - comparison with blood and oral fluid analyses.

Oratect^R

Oratect^R was selected because the device could be used onsite without any instrument for reading the results. Some tests were received for preliminary evaluation, which were evaluated in comparison with a representative from the police who had experience from ROSITA I. The experience was very promising. The test was user-friendly with few steps, the results could be read within five - ten minutes. The lines that appeared indicating positive or negative results were easily readable without any need for an instrument. Information of that BZDs would probably be included later in the drug screening program, increased our interests. More devices were ordered for police training and testing of suspected drugged drivers.

The new batch of the devices seemed to be different from the first. During training of the police, it was difficult to get sufficient oral fluid on the collection pad, even after keeping the device in the mouth for much longer time than recommended by the instructions. In some cases, oral fluid was absorbed on only half of the collection pad. The problems resulted in several invalid test results. A new batch was ordered from the company, but the same problems occurred. Still, the police started to evaluate the devices testing suspected drugged

¹ Not included for ROSITA comparison

drivers, combined with collection of oral fluid and blood samples as described earlier. However, many tests results were invalid, which was not motivating for the police. It was decided that the devices should not be used before new batches could be delivered that seemed more convincing and without invalid test results. We were later informed that the producer had changed the product between the different batches that were delivered.

Comparison of Oratect^R devices with oral fluid and blood samples analyses.

Due to the problems described earlier, only ten pairs of device, blood and oral fluid for comparison were obtained. At least twenty out of thirty Oratect^R tests were reported as invalid. The value represents a minimum, as some invalid tests were not recorded. In some cases, two or three tests were tried on one subject, which all showed invalid results. The devices showed few false positive or false negative results compared with blood and oral fluid analyses as shown in table 2. However, the number of available results is too low to draw any conclusion. If the devices will be changed, a new evaluation has to be performed anyway.

Table 2

Comparison of results from Oratect^R devices (n=10) with blood and oral fluid analyses.

	Amphetamines		Cannabis		Opiates		Cocaine	
	Blood n=10	Oral flu. n=8	Blood n=10	Oral flu. n=8	Blood n=10	Oral flu. n=8	Blood n=10	Oral flu. n=8
TP	4	2	0	0	3	5	1	1
TN	5	5	8	6	3	3	9	7
FP	0	0	1	1	2	0	0	0
FN	1	1	1	1	2	0	0	0
Prevalence	50	38	10	13	50	63	10	13
Sensitivity	80	67	0	-	60	100	100	100
Specificity	100	100	89	86	60	100	100	100
PPV	100	100	0	-	60	100	100	100
NPV	83	83	89	86	60	100	100	100
Accuracy	90	88	80	75	60	100	100	100
Invalid	20*							
Denied	4							

*Minimum value

Definitions:

TP= True positive

TN= True negative

FP = False positive

FN = False negative

Prevalence: TP+FN/n (%)

Sensitivity: TP/(TP+FN) (%)

Specificity: TN/(TN+FP) (%)

Positive predicted value (PPV): TP/(TP+FP) (%)

Negative predicted value (NPV): TN/(TN+FN) (%)

Accuracy: (TP+TN)/n (%)

Comments from the police

The first batch of devices seemed user-friendly, easy to use with few steps before the results could be read. The time before reading of the results should be shorter. BZDs were not included. Outdoor testing was not performed. The devices cannot be used because of too

many invalid results.

DrugTest^R

DrugTest^R from Dräger was selected to get experience with a device using an electronic reader. Training was performed by representatives from Dräger, Germany, both at DFTDA and at the different police stations. The testing procedure included many different steps requiring more than 15 minutes for the total testing process, including four minutes reaction time before the electronic reader, followed by eight minutes development in the reader. Even with rather good instruction, it was easy to make a mistake resulting that the process had to start again, including extra eight or twelve minutes before the final results. Several invalid results were obtained, which were not reported. The reader could only be used on the police station and was not suitable for roadside use in Norway. Based on comments from the police, it was necessary to get sufficient time for training, several tests were needed before they were familiar with the system, which was not possible to organise during the project time. In several cases, a police officer without sufficient training was on duty when a suspected drugged driver arrived for blood sampling and clinical examination. BZDs were not included, which are important for Norwegian traffic control.

Unfortunately, few tests using DrugTest^R were performed, due to the time-consuming, complicated test procedure and that the electronic reader could not be used outside. The available results from comparing the devices, blood and oral fluid analyses are presented in table 3. As seen from the table, the number of results from oral fluid analyses is lower than blood results, due that some drivers refused to give oral fluid sample. Few false positive and few false results were obtained when comparing both biological matrices. The number of results available is too low to draw any conclusion. The company has informed that this product has been withdrawn from the market and that a new product for onsite testing will be developed.

Table 3.

Comparison of results from DrugTest^R devices with blood (n=14) and oral fluid (n=9) analyses.

	Amphetamines		Cannabis		Opiates		Cocaine	
	Blood n=14	Oral flu. n=9	Blood n=14	Oral flu. n=9	Blood N=14	Oral flu. n=9	Blood n=14	Oral flu. N=9
TP	7	4	0	0	5	5	1	0
TN	5	2	12	6	8	4	11	8
FP	1	1	0	0	1	0	0	0
FN	1	2	2	3	0	0	2	1
Prevalence	57	67	14	33	36	56	21	11
Sensitivity	88	67	0	0	100	100	33	0
Specificity	83	67	100	100	89	100	100	100
PPV	88	80	0	-	83	100	100	-
NPV	83	50	86	67	100	100	85	89
Accuracy	86	67	86	67	93	100	86	89
Invalid *	3							
Denied	5							

*Minimum value

Definitions: See table 2

Comments from the police

The testing procedure was too complicated with many steps. Sufficient training was necessary, which is difficult with many different police officers on duty. At least 15 minutes before the results are available, is too long time. BZDs should be included in the drug program. The device was not acceptable for pre-screening of suspected drugged drivers in Norway.

Oraline^R

Oraline^R was selected because the device can be used onsite without any instrument to read the results. The devices were mostly used outdoors during roadside control and for testing of drug addicts. In most cases only oral fluid could be collected for comparison. Most of the test subjects were willing to participate. The procedure was simple, including few steps before the results could be read. In several cases it was difficult to get sufficient sample volume because the drug abusers frequently have a dry mouth. Spitting in a cup was recommended by the instruction, but not very hygienic. The frequency of invalid tests was rather high (n=15) compared to 23 successful. The reason may be insufficient sample volume and that some tests were collected outdoors during heavy raining (See comments from the police). The time from sampling to final results was up to 10 minutes, which is higher than the requirements from the police. BZDs were not included in the drug program.

Comparison of the results with oral fluid and blood samples analyses.

The results for comparison are shown in table 4. Only five blood samples were collected in addition to 23 oral fluid samples. Totally, results from the devices were approximately in good accordance with the oral fluid analyses. However, few samples were positive for cannabis, cocaine and amphetamines. The number of available results is too low to draw any final conclusion.

Table 4.

Comparison of results from Oraline^R devices with blood (n=5) and oral fluid (n=23) analyses.

	Amphetamines		Cannabis		Opiates		Cocaine	
	Blood n=4	Oral flu. n=22	Blood n=4	Oral flu. n=22	Blood n=4	Oral flu. n=22	Blood n=4	Oral flu. n=22
TP	1	5	0	1	2	13	0	0
TN	3	15	4	18	1	6	4	19
FP	0	0	0	0	1	1	0	2
FN	0	2	0	3	0	2	0	1
Prevalence	25	32	-	18	50	68	0	4,6
Sensitivity	100	71	-	25	100	87	-	0
Specificity	100	100	100	100	50	86	100	90
PPV	100	100	-	100	67	93	-	0
NPV	100	88	100	86	100	75	100	95
Accuracy	100	91	100	86	75	86	100	86
Invalid	15							
Denied	0							

Definitions: See table 2.

Comments from the police

The test procedure for Oraline^R was simple with few steps. A drawback was that a rather large sample volume was needed which may be difficult for drug abusers. It seemed that the time for the sample to move from collection area was dependent on the temperature. Cold weather (approximately 0⁰ C) during the testing period increased the time for the sample to move from collection pad to the reaction area with antibodies. The lines indication positive or negative results were often too pale for clear conclusion. Some tests were performed during heavy raining, resulting that raindrops came into the reaction area. It was then impossible to read the results. A transparent protective cover would solve this problem. The time from sampling to final results was too long to be used for roadside control. BZDs were missing. The boxes keeping the devices stored plastic cover were unnecessary. The testing instructions could be printed on the plastic cover instead of the boxes.

Drugwipe^R

Drugwipe^R was also evaluated in Norway during ROSITA - 1. At that time, amphetamines and opiates could only be detected using two different devices. Changes and improvement have occurred since that time. Four different drugs/drug groups have been included in one test (amphetamines, cannabis, opiates and cocaine). A single test has been developed for BZDs. As far as we know, this is the only available device that can detect BZDs. The devices were therefore important to evaluate for Norwegian use. The equipment was easy to use and the results could be read within few minutes. Some tests were performed during a roadside control. All drivers who were asked accepted to participate. The devices were also used to test drug addicts who were willing to participate and further told which drugs they had used. This information was mostly in accordance with the results from oral fluid analyses. One serious problem with Drugwipe^R was the very pale, pink lines indication positive results, which were very difficult to read. Some lights or reader was promised, but arrived when the project was finished. The pale lines may be the reason for both false positive and negative results. A small water container connected to the devices would be preferable instead of the water bottle, to avoid possible contamination.

Comparison of results with oral fluid and blood samples analyses.

Table 5 shows the results from the devices combined with blood and oral fluid sample analyses. Too many false positive results were obtained, particularly for opiates where a better correlation during ROSITA-1 on a single drug device, was obtained. Several opiate tests from heavy drug abusers who had used heroin, showed negative results, while high concentrations of morphine, 6-MAM and codeine were detected in the corresponding oral fluid samples. For cannabis and BZDs, the number of false negative results was not acceptable. Better sensitivity for these drugs is necessary. Some of the false positive or negative results may be due to misinterpretation when reading the very pale colour lines. In some cases, high concentrations of morphine and 6-MAM detected in the oral fluid (due to heroin use), resulted in false negative amphetamine and BZDs results on the devices, while these compounds were detected at high concentrations in oral fluid.

As seen from the confirmation results in blood and oral fluid, BZDs were the most frequently detected drugs. It is therefore important that future devices cover this drug group with sufficient sensitivity, including low-dose BZDs.

Comments from the police

The testing procedure was easy with few steps. It is positive that the BZDs are included in the drug testing program. All drug groups group on one device would be easier and less time consuming. However, an indistinct colour change line made the reading very difficult, particularly outdoors with poor lighting. This problem may have resulted in wrong conclusion of positive or negative results. Increased contrasts with more distinct colour line (blue or

black) are necessary. A small and user-friendly reader could be favourable but is not the primary demand. Distinct colour changes would be the best for practical use. Testing during heavy raining made the reading even more complicated with raindrops in the reaction area. A transparent protective cover would solve this problem. The water bottle makes the devices less user-friendly and not practical during cold wintertime. Contaminations of the water may occur and therefore influence on the results. The situation may give good arguments for a lawyer if a driver is taken to a police station for blood sampling, based on false positive results. An incorporated sealed container in the package with sufficient fluid that also works below zero degree temperature would be preferable.

Table 5.

Comparison of results from onsite testing using Drugwipe^R devices compared with blood and oral fluid confirmation analyses.

	Amphetamines		Cannabis		Opiates		Cocaine		Benzodiazepines	
	Blood n=22	Oral flu. n=66	Blood n=22	Oral flu. n=66	Blood n=22	Oral flu. n=66	Blood n=22	Oral flu. n=66	Blood n=22	Oral flu. n=48
TP	7	15	1	3	4	14	0	0	1	18
TN	14	43	14	44	14	31	19	63	8	14
FP	1	2	0	8	2	1	1	1	2	5
FN	0	6	7	11	2	20	2	2	7	11
Prevalence%	32	32	36	21	27	52	9	3	44	60
Sensitivity	100	71	13	21	67	41	0	0	13	62
Specificity	93	96	100	85	88	97	95	98	80	74
PPV	88	88	100	27	67	93	0	-	33	78
NPV	100	88	67	80	88	61	90	97	53	56
Accuracy	95	88	68	71	82	68	86	95	50	67
Invalid	4								5	
Denied	5								5	

Definitions: See table 2

Comparing results from blood and oral fluid analyses

Comparing results from blood and oral fluid analyses in cases where both matrices were available showed rather good correlation. In general, drugs that were detected in blood samples were mostly also detected in oral fluid except for some deviations which may be explained from the following reasons: i) the different cut-off limits used for blood and oral fluid ii) the sampling procedure iii) the results were not correlated for the variable oral fluid volumes that was collected and further diluted with the buffer in the collection devices (Intercept^R) (many drugs users have very dry mouth) iv) passage of drugs from blood to oral fluid may be variable (9). It is not known if low sample volumes always have higher drug concentrations in cases of dry mouth, compared to a “normal” situation. Volume correlation may not give the “correct” value.

Examples of blood/oral fluid comparison of some selected drugs are illustrated in figure 1, e.g. amphetamines, THC, morphine and diazepam. All cases where amphetamine was detected in blood were also detected in the corresponding oral fluid samples, while four samples were positive for amphetamine in oral fluid but not in blood. It has been documented earlier that amphetamine concentrations in oral fluid/saliva are higher than in blood (9). Diazepam was detected in some blood samples, however not found in the corresponding oral fluid samples using the recommended cut-off limits. A similar pattern was also found for other BZDs due to that the concentrations in oral fluid/saliva are normally lower compared to

blood samples (10). Based on validation of our oral fluid LC/MS/MS-method, lower cut-off limits than recommended for ROSITA-samples can be used for BZDs and some other compounds. Using lower cut-offs could have given a better correlation between some pairs of blood and oral fluid samples.

Comparison of drug detections in blood and oral fluid

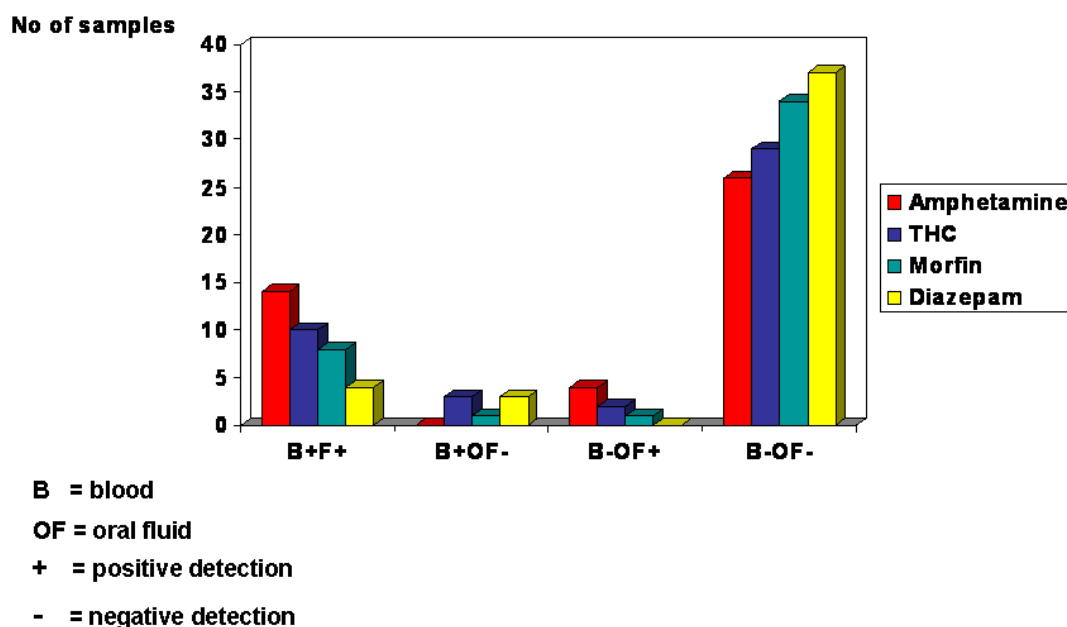


Figure 1: Examples of blood/oral fluid comparison of some selected drugs

Final comments and conclusion

The number of tests with corresponding pairs of devices, blood and oral fluid samples, were lower than expected from the beginning of the project. Several reasons were responsible for this problem; some devices showed too many invalid tests, too long time to wait from sampling to final results including many steps included in the procedure, an electronic reader not suitable for roadside use in Norway. Many police officers were involved in traffic control in the participating police districts. It was not possible to give all the necessary training during the project period. Several oral fluid samples were collected during random roadside control, using devices (Drugwipe^R, Oraline^R) suitable for such type of control. However, most of these drivers had not used any drugs and very few positive results were obtained for comparison. So, far the available devices are not acceptable for pre-screening of suspected drugged drivers in Norway. Since one or more drugs are found in approximately 80% of the apprehended drivers without any drug device available, the effectiveness of possible new equipment is important. The requirements described from the police for devices to be used for roadside testing, including user-friendliness, time from sampling to final results which is easy to read based on with distinct colour change. BZDs included in the drug program are mandatory. Drivers that probably are released on wrong decision due to insufficient evidence by the present roadside control (DRE-program) are important for extended investigation, probably using an onsite drug test. However, good correlation (better than the tested devices) between the results from onsite screening and blood analyses is necessary with increased sensitivity for cannabis and BZDs.

The Norwegian authorities represented by the Ministries of Police, Transport and Health want

to prioritize equipment for road -side screening as a part of increased action against drugged driving and to simplify the police work. New devices that satisfy the requirements from the police including good correlation with blood samples are welcome. Onsite devices are especially mentioned in the proposal from a working group appointed by the Ministry of Transport. The aim is equipment with similar user-friendliness and time use as an alcoholmeter, for testing of suspected drivers.

Several police officers not involved in the projects have asked for the devices to be used not only for roadside control, but also for other criminal cases where drugs are suspected.

Equipment for pre-screening using oral fluid, are also interesting for testing in other situations, e.g. drug addicts in treatment programs, social welfare cases and other cases where drug testing is discussed. Important is the more acceptable sampling procedure compared to urine drug testing. Requests and interests from other institutions involved in drug testing are frequently received.

Acknowledgements

The project group appreciates collaboration from the following police districts:

Oslo Police District, Rogaland Police District and Bjørn Steinar Sjøthun, Asker and Bærum Police Districts and Roar Bjartan, S. Buskerud Police District and Henning Skau. We also appreciate support from Police Mobile Force during random roadside control. Special thanks to the staff at DFTDA and mainly to Terje Hammer for illustration of device instructions and the protocol, Elisabeth L. Øiestad, Unni Johansen and Wenche Andresen for method development, validation and analyses of oral fluid samples. Finally, thanks to Division Director Jørg Mørland, responsible for the project, for valuable advice and interest.

Appendix: Drugwipe results using a flashlight

As the results could be difficult to interpret due to pale and indistinct colour lines, some small flashlights were received from the Norwegian Drugwipe representative (Defendo AS, Norway). Readers were not available from the producer. Two different police officers tested drug users who were willing to participate. The tests were performed on the street and oral fluid was collected simultaneously for confirmation analyses. For interpretation of results, the police flashlight was better than the small flashlights received from the company.

One main problem during this testing was the temperature during wintertime in Norway that was below -5°C , resulting that the results could not be read before 10 – 15 minutes after sampling. Some of the tests were invalid, e.g. one part of the devices were invalid with no control line (amphetamines, cannabis), while the control line appeared on the other part of the test. After the tests were brought to the police station, some former invalid results could be read. The tests were brought to our Institute and controlled after 5 days. This time more of the former invalid results could be read. When comparing Drugwipe results with oral fluid confirmation, both false negative and “correct” results were obtained. The results read after five days showed best accordance with the confirmation analyses. The results from these tests have not been included in the table (table 5) together with the other tests results.

Conclusion: Using an intense flashlight may be helpful for interpretation of results, but the contrast of the colour line is not satisfactory. The devices cannot be recommended for temperature below zero as time from sampling to available results increase significantly (10 – 15 min or more), showing several invalid results. When the devices are stored at room temperature for one or more days, some control lines may appear and the results can read. The conclusion is that Drugwipe devices are not useful for road-side testing during wintertime

when the temperature is below zero.

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 folkehelseinstituttet Divisjon for retts toksikologi og rusmiddelforskning Postboks 4404 Nydalen 0403 Oslo Besøk og prøvelevering: Lovisenberggt. 6 Sentralbord 23 40 78 00 Telefaks 22 38 32 33	ROSITA II- PROSJEKT Testresultater spyttprøver
	OPPLYSNINGER OM PRØVEGIVER Navn _____ (etternavn) (fornavn) Fødselsnr. Kjønn: M ☐ K ☐

ROSITA-prosjektet (ROAd Side Testing Assessment) er et felles europeisk og amerikanske prosjekt som skal undersøke tester for andre rusmidler enn alkohol før eventuell blodprøvetaking. I Norge skjer dette i form av et samarbeid mellom enkelte politidistrikter og Divisjon for rettstoksikologi og rusmiddelforskning, Folkehelseinstituttet. Flere tester skal utprøves.

**Testresultatet i spyttprøven er en screening-analyse, og resultatet er ikke et juridisk bevis.
SE INFORMASJON NESTE SIDE**

TESTSYSTEM	STOFFGRUPPE	TESTRESULTAT		
		pos	neg	ikke egnet
ORALINE SUM BIOMEDICAL	AMFETAMIN/ METAMFETAMIN	☐	☐	☐
	THC (cannabis)	☐	☐	☐
	OPIATER	☐	☐	☐
	KOKAIN	☐	☐	☐
DRUGWIPE SECURETEC	AMFETAMIN	☐	☐	☐
	METAMFETAMIN	☐	☐	☐
	THC (cannabis)	☐	☐	☐
	OPIATER	☐	☐	☐
	KOKAIN	☐	☐	☐
	BENZODIAZEPINER	☐	☐	☐
☐ ØNSKET IKKE Å AVGI SPYTTPRØVE				

PRØVE TATT:

☐ UTE

☐ INNE

☐ IKKE GODKJENT TESTRESULTAT

Skjemaet sendes til Folkehelseinstituttet/RE sammen med undersøkelsesprotokoll og blod/urinprøve.
Rekvirenten tar kopi.
Kontaktperson ved FHI/RE: Asbjørg S Chistophersen tlf 23407860/ Terje Hammer tlf 23407880

Sted og dato

Underskrift prøvetaker

ROSITA-II Utprøving av spytttester for primærundersøklese av rusmiddelinntak

Generelt

- Testene ("on-side-test") brukes ved mistanke om påvirkning av andre rusmidler enn alkohol (trafikk/andre straffbare forhold), som blir fulgt opp med blod-/urinprøver (utvidet prøve).
- 2 tester skal evalueres i den første av to prøveperioder : DRUGTEST (Dräger) og ORATECH (Branan). To andre tester i 2005.
- Utfør testing på politistasjonen, i tilknytning til blodprøvetaking, eller på veien, hvis hensiktsmessig.
- Det skal også tas spyttprøve (oppsamlingsutstyr INTERCEPT -OraSure), som sendes sammen med blod-/urinprøve/ protokoll (utvidet prøvesett) til Div. for rettstoks. og rusmiddel- forskn., Folkehelseinst. (Rettstoks). Spyttprøven skal brukes til bestemmelse av rusmiddelmengde med spesifikk metode og sammenlignes med primærttestresultater.

Prosedyre

- Prøvegiver forespørres om han/hun er villig til å avgi spyttprøve. **Prøven er til forskningsformål og vil ikke ha betydning for det videre utfall av saken.**
- En av testene velges ut. Følg bruksanvisning for testen. Dersom prøvegiver er villig kan det tas prøve med begge tester.
- Bruk engangshansker.
- Resultater, prøvegiverinformasjon, dato osv., registreres registreres på ROSITA-skjema, utarbeidet for prosjektet. Kryss også av hvis resultatet blir "Ikke godkjent test".
- Deretter samles spyttprøve for analyse ved Rettstoks. ved hjelp av oppsamlingsutstyret. Følg instruks. Prøveglasset merkes med strekkodeetikett fra protokollen for utvidet prøve som skal følge blodprøve, event navn og prøvetakingstidspunkt, egent etikett
- Merket spyttprøve og ROSITA-skjema sendes til Rettstoks., se punkt over.

Vurdering av utstyret

- Noter gjerne positive/negative egenskaper med utstyret. Dette er viktig informasjon, som vil bidra til utvikling av utstyr som er hensiktsmessig til bruk for politiet, samtidig som det kan avsløre hvilke stoffer som er tatt inn.
- Resultater fra sammenligning av utstyret og analyse av spytt/blodprøver vil blir presentert for når prosjektet er avsluttet.

TAKK FOR HJELPEN!

Chapter 8: EVALUATION OF ROADSIDE ORAL FLUID DRUG TESTING DEVICES

M. López-Rivadulla, A. Cruz, O. Quintela, A. de Castro and M. Concheiro

Forensic Toxicology Service. Institute of Legal Medicine. University of Santiago of Compostela. Spain.



ABSTRACT

This report presents a collaborative study with the Spanish Traffic Police to assess the effectiveness of oral fluid drug detection technology to detect onsite drug abuse by drivers. The project was conducted in areas from the Northwest of Spain (Galicia) by teams composed of scientists working in collaboration with traffic police. Two different models of oral fluid devices were tested during weekends in 2004 and 2005 in areas near discotheques and sites of leisure. Approximately 500 drivers were selected at Police controls. They agreed to participate through informed consent. Saliva samples were collected by spitting and sent to the Laboratory to confirm devices results. The results for each device, and practical evaluation from Police officers are presented. Also, comparison between devices and laboratory results are showed.

INTRODUCTION

Nowadays the Spanish police does not have equipment for onsite drug testing. The members of the Forensic Toxicology Service, belonging to the Institute of Legal Medicine of the University of Santiago de Compostela (ILMUSC), established a collaborative agreement with the Traffic Directorate in Galicia (NW of Spain) to assess the utility of onsite devices and determine the feasibility of their use by police force agents for testing drivers. This agreement was developed in a similar context as during the ROSITA I project in 1999/2000. That was:

- The restriction to a specific geographic area (NW Spain).
- Its voluntary nature

Before the project started we applied for permission to the Director of Public Prosecutions to accomplish the ROSITA II with traffic Police forces. Two different criteria related ROSITA I were pointed out:

- Training of devices should be covered by manufactures
- The devices should be used for drivers who admitted having used drug in the last 6 hours.

A training program for police agents was organized at the Institute of Legal Medicine of the University of Santiago de Compostela (ILMUSC) in collaboration with manufacturers. The police officers were trained in the handling of the devices in groups of 10 people. Instructions how to use the equipment was available from the manufacturers and further adapted in collaboration with the ILMUSC members. A simple manual was designed to facilitate the correct use of the device by the police officers. In addition the research team designed a protocol to be used for each case, to record sex, age, date of sampling, results. This protocol was filled by the police officers and incorporated to final report (Appendix A)

In order to proceed to a correct evaluation of the devices, we obtained samples from drivers and also from passengers, admitting recent drug use. This situation was more remarkable in the second phase of controls, and during the use of second device (Dräger device). In Spain it is not possible at the moment to collect a blood sample for the analysis of drugs without the consent of the drivers; therefore we only have been able to carry out the tests in oral fluid.

METHODS

Subject selection, organisation, location of the checkpoints.

The selection was done randomly and always carried out by the Traffic Police. The agents made vehicles stop and asked the driver to collaborate by undergoing -always voluntarily and without any kind of adverse consequences for him or her- the tests with the studied devices. On the other hand, the driver did not need to give his or her name and address, in order to ensure that the test was totally anonymous.

The conditions for the performance of the controls of ROSITA depended on the planning of the Traffic Police. In this context all the controls were done during the Friday and Saturday nights, from 1 a.m. to 8 a.m. and located in areas near to discotheques. Figure 1 shows the sites of the drug controls. The population that has been the object of the controls of the ROSITA project is located in a big area of the Northwest of Spain. The group of the potential population reaches 705.000 inhabitants, with a slight dominance of the female sex over the male one. It is reckoned that 56% of all these inhabitants have driving licences and 39% of them are women whereas 61% are men. The fieldwork was carried out during November and December 2004 and January to July 2005. Figure 2 shows the age and sex of the drivers.



Figure 1: sites of the drug controls

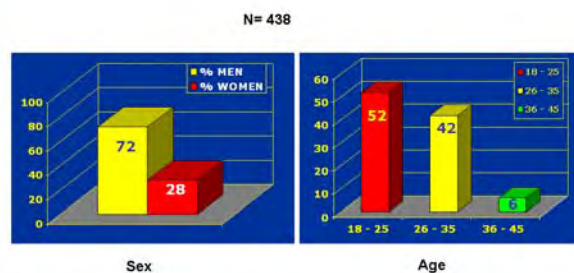


Figure 2

Figure 2: Sex and age distribution of the subjects

The practical use of the devices by the Police officers

Fortunately both devices used in the fieldwork were different from the devices used in ROSITA I. Oral fluid is well accepted as a screening matrix by the police officers. They prefer equipment that is comparable to the time use and easiness to the breath alcohol test. Also they want a simple to interpret device with an electronic reader. In general terms they had no problems to work with both tests after a period of habituation, although they prefer a device that does not require a subjective interpretation. The duration of the test is not a critical condition, but the precision and reliability are highly prized for them. Failures in the device reader or function are one the more important disadvantages valued by the police.

FIELD WORK.

Devices Assigned: We have tested two devices. The On-Site Ansys Varian OraLab and the Dräger devices.

All officers were trained at the beginning of the collection period on the operation of the oral fluid drug testing devices. For each device scientific personnel belonging to the manufacturers explained the characteristics of the procedure. The administrative procedures to be used (i.e. consenting of the subject and data collection/recording) were explained by the personnel from the Institute of Legal Medicine.

On-Site OraLab is a simple, qualitative assay that detects recent drug use in oral fluid. Its uniquely designed container automatically splits the specimen, using half for immediate drug-of-abuse testing and storing the remaining sample for confirmation testing, if required. This device is described in the figure 3.

Anslys VARIAN ORALAB



Figure 3

Figure 3: Varian Oralab.

Drugs detected: Amphetamines, Cocaine, Methamphetamine, Opiates, THC, and PCP.

In order to obtain the sample, the police officer instructs the driver to collect saliva by placing the collector foam inside mouth for three minutes, and completely saturates the collector foam with saliva; no chewing, compressing, or sucking on the collector foam are allowed. The OraLab cassette is placed on a flat surface and the confirmation well cap is lifted to assure that the flow of saliva into the well is not obstructed. The collection foam is removed from the mouth. It should be very wet when removed from the mouth, and must not be compressed when removed. The collector foam is placed vertically into the sample well on top of the device, and the collector is pushed slowly (approximately 5 seconds) and firmly downward until it comes to a stop. Saliva flows directly into the test cassette. Test validity and results must be interpreted between 10 and 15 minutes after initiating the test. A valid test is indicated by a band in the test valid window. An invalid test is indicated by the absence of a distinct band in the test valid window or by a reddish background in the results or test valid windows which obscures the presence of bands 15 minutes after the test is initiated. The test results are interpreted immediately as either negative or preliminary positive; A preliminary positive result for a given drug (i.e., drug present above the cutoff, suggesting current drug use) is the absence of a band in the test result window adjacent to the drug name, and a negative result (i.e., drug absent or below the cutoff) is the presence of a band in the results window adjacent to the drug name. Table 1 shows the cutoff limits for this device. The interpretation of the results is visual. The device permits saving a specimen for confirmation (The wick can be separated from the device and sent to the lab).

Table 1: Cut-offs of the Varian OraLab.

ORALAB CUTOFFS

MORPHINE	40 ng/mL
COCAINE	20 ng/mL
D-AMPHETAMINE	50 ng/mL
D-METAMPHETAMINE	50 ng/mL
THC	100 ng/mL

In a second phase we tested the Dräger Drug test. This system is a competitive immunoassay for the detection of drugs in oral fluid. That test kit includes an oral collection device and a cassette containing a lateral flow strip that has been impregnated with test and reference lines. Several warnings and precautions occur in the preparation of test regarding the use of material provided.

Drugs detected: Amphetamines, Cocaine, Methamphetamine, Opiates, and THC.

The collection device is inserted between the cheek and the gum and swabbed side to side in the mouth until the collector is saturated. This operation takes about 1-2 minutes. After, it is necessary to strictly follow the instructions for use of device:

The buffer cartridge is inserted into the lower groove of the test cassette by aligning the lower tabs on the cartridge with notches on the cassette. The cap is rocked and removed.

The sample collection device is inserted into the buffer cartridge and pressed down on the sample collection device (notice that the sample collection device clicks slightly into place and a cracking sound can be heard when it is pushed down firmly).

The handle of the sample collection device is removed by means of an anticlockwise quarter turn.

The buffer cartridge is closed and it incubates during 4 minutes.

The buffer cartridge is turned clockwise until the upper groove is positioned above the groove of the test cassette. The buffer cartridge is pushed down firmly until the cartridge wings touch the edge of the cassette. The fluid is allowed to develop for 8 minutes.

After 8 minutes the sample ID is entered in the analyzer and the cassette is inserted into the analyzer.

The analyzer will indicate that the door is closed and read cycle has begun. The analyzer will report results in 4 minutes.

Finally the machine displays the results in the screen for each drug with a “+” or “–”. Also all results are saved automatically into memory. This device is described in the figure 4. Table 2 shows the cutoff limits for this device.

Table 2: Cut-offs for the Dräger DrugTest

DRÄGER CUTOFFS



Figure 4: Dräger Drug test

OPIATES	40 ng/mL
COCAINE	5 ng/mL
AMPHETAMINE	50 ng/mL
METAMPHETAMINE	10 ng/mL
THC	20 ng/mL

In both phases, oral fluid was collected by spitting in a dry polypropylene tube, and it was sent to Laboratory for analysis and stored frozen (-20°C). The sample was the same as that of the driver tested in the control.

LABORATORY CONFIRMATION PROCEDURES AND METHODS

All analytical procedures regarding confirmation process of drugs involved in the project were done by Liquid Chromatography coupled with Mass Spectrometry (LC-MS). We have developed and fully validated, two simple and low-cost LC-MS methods; one for the simultaneous determination of morphine, 6-monoacetylmorphine (6-MAM), amphetamine, methamphetamine, MDA, MDMA, MDEA, MBDB, benzoylecgonine and cocaine in plasma and oral fluid, after solid phase extraction (SPE), using 400 µL of oral fluid; the other one designed specifically to quantify THC in 0.2 µL of Oral fluid after liquid-liquid extraction. Both procedures have been published (1, 2, 3). Table 3 shows the LOQ limits for each drug applying this procedure.

We used the cut-offs decided in Strasbourg for the definitions of a positive/negative in oral fluid or blood (Table 4).

In the THC detection, Liquid Chromatography was performed by using a Waters Alliance 2795 separation module system. Chromatography was carried out using a XTerraMS C18 column (2.1 x 100 mm, 3.5µm), eluted isocratically using 0,1% formic acid and acetonitrile (15:85), delivered at a flow of 0.25 mL/min. Data acquisition was controlled using MassLynx NT 3.5 software.

Regarding other drug, chromatographic separation was performed with an Atlantis dC18, 3 µm (100 x 2.1 mm i.d.) reversed-phase column. The mobile phase, delivered at a flow rate of 0.2 ml/min at 26°C, was a gradient of acetonitrile and a pH 3.0 ammonium formate buffer,

programmed as follows: 5% acetonitrile during 1 min, linearly increased to 50% in 10 min, kept that percentage for 1 min, decreased to 5% (original conditions) in 1 min and equilibrated for 4 min, which resulted in a total run time of 17 min.

Table 3: LOQ for each drug

Substance	<i>LOQ oral fluid (ng/mL)</i>
Amphetamine	2
Methamphetamine	2
MDMA	2
MDA	2
MDEA	2
Cocaine	8
Benzoyllecgonine	8
Morphine	2
Codeine	2
THC	2
THCCOOH	
11-OH-THC	
Diazepam	0.5
Nordiazepam	0.5
Temazepam	0.5
7-aminoflunitrazepam	2
Bromazepam	2
Lorazepam	0.5
Clonazepam	
(Zopiclone/Zolpidem)	

In both cases, the detection was performed by using a Micromass ZMD 2000 mass spectrometer (Micromass, Manchester, UK) fitted with a Z-spray ion interface. Ionisation was achieved by using electrospray in the positive ionisation mode (ESI⁺). Nitrogen was used as nebulisation and desolvation gas. To optimise ionisation and ion transmission conditions for each compound and for the IS, separately 5 µL of a 10 µg/mL solution in the mobile phase were injected without HPLC separation into the ion source. In order to obtain the highest possible intensity for quantitation and confirmation ions, the fragmentation energy (cone voltage) was optimised. During this experiment, a mass range from m/z 100 to 400 was monitored in SCAN mode, applying different cone voltages. Acquisition was made in the selected ion-monitoring mode (SIM). For the quantification of each compound, the protonated molecule [M+H]⁺ was selected as the quantifier ion and one main fragment was selected as the confirmation ion. In the case of deuterated IS only the protonated molecule was selected.

Table 4: cut-off used

Substance	LOQ blood (ng/mL)	LOQ oral fluid (ng/mL)
Amphetamine	20	25
Methamphetamine	20	25
MDMA	20	25
MDA	20	25
MDEA	20	25
Cocaine	20	8
Benzoyllecgonine	20	8
Morphine	10	20
Codeine	10	20
THC	1	2
THCCOOH	5	
11-OH-THC	1	
Diazepam	50	5
Nordiazepam	50	5
Temazepam	50	5
7-aminoflunitrazepam	1	2
Bromazepam	10	5
Lorazepam	10	5
Clonazepam	5	5
(Zopiclone/Zolpidem)	10	10

RESULTS.

General Overview.

Within the period from November 2004 to July 2005 approximately 500 oral fluid devices were used by 20 different police officers to test 438 drivers. Figure 2 shows the global statistical data concerning the total number of drivers, and distribution according to age range and sex.

Practical assessment by the police officers.

As it was the first time that many police officers made tests with biological fluids, it is necessary to emphasize that they made it with enormous professionalism. All it in spite of having to handle a biological fluid like the oral fluid, and to handle an apparatus that to a certain extent presents a certain complexity. In addition, differences of evaluation for both devices have existed; the ORALAB device was much simpler to handle, whereas the Dräger demanded a more exhaustive control in the different steps to carry out the test. Nevertheless the interpretation of the disappearance of the line in the ORALAB, was rejected as opposed to the printed result that appeared in the screen of the Dräger. The police officials very categorically reject a subjective interpretation of the test. Another negative aspect to consider is when the test fails in its final stage. The police officers confront this situation with certain disappointment, therefore it is essential to know the reasons for which the test does not work and to diminish the possibilities that it happens. The traffic agents do not demand an exaggerated rapidity in the development of

the test; they know that it cannot be like the test of the alcohol, but they wish that it is most similar. Figures 5 and 6 show the number of failures detected with each device.

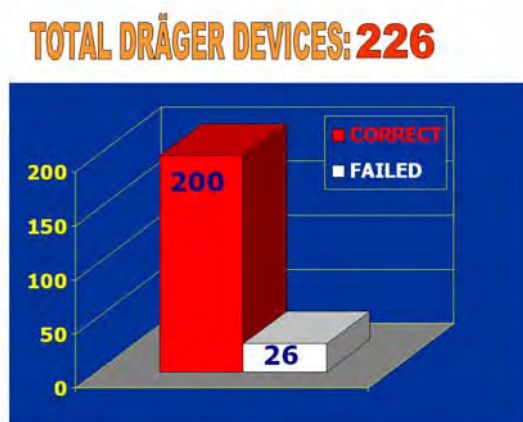


Figure 5: number of failures with DrugTest

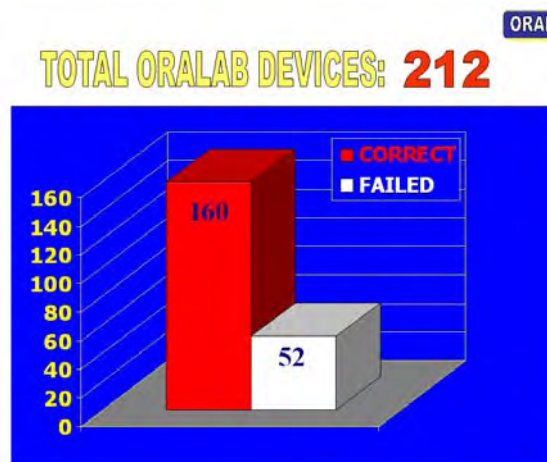


Figure 6: number of failures with Oralab

Table 5: Results for cannabis detection with OraLab.

ORALAB -THC			
N		158	
True positives		14	
True negatives		141	
False positives		2	
False negatives		1	
Sensitivity		93,3	
Specificity		98,6	
Positive predictive value		87,5	
Negative predictive value		99,3	
Prevalence		9,5	
Accuracy		98,1	

THC		MS result	
		+	-
On-site result ORALAB	+	14	2
	-	1	141

Table 6: Results for cocaine detection with OraLab.

ORALAB -COCAINE	
N	173
True positives	32
True negatives	138
False positives	3
False negatives	0
Sensitivity	100
Specificity	97,9
Positive predictive value	91,4
Negative predictive value	100
Prevalence	18,5
Accuracy	98,3

COCAINE		MS result	
		+	-
On-site	result	+	32
ORALAB		-	0
			138

Table 7: Results for methamphetamine detection with OraLab.

ORALAB - METHAMPHETAMINE	
N	158
True positives	0
True negatives	157
False positives	1
False negatives	0
Sensitivity	-
Specificity	99,4
Positive predictive value	-
Negative predictive value	100
Prevalence	0
Accuracy	99,4

METHAMPHETAMINE		MS result	
		+	-
On-site	result	+	0
ORALAB		-	0
			157

Table 8: Results for amphetamine detection with OraLab.

ORALAB - AMPHETAMINE	
N	173
True positives	1
True negatives	170
False positives	1
False negatives	1
Sensitivity	50
Specificity	99,4
Positive predictive value	50
Negative predictive value	99,4
Prevalence	1,2
Accuracy	98,8

AMPHETAMINE		MS result	
		+	-
On-site	result	+	1
ORALAB		-	170

Table 9: Results for opiate detection with OraLab.

ORALAB - OPIATES	
N	173
True positives	6
True negatives	167
False positives	0
False negatives	0
Sensitivity	100
Specificity	100
Positive predictive value	100
Negative predictive value	100
Prevalence	3,5
Accuracy	100

OPIATES		MS result	
		+	-
On-site	result	+	0
ORALAB		-	167

Table 10: Results for cannabis detection with Dräger.

DRAEGER -THC	
N	256
True positives	58
True negatives	141
False positives	16
False negatives	41
Sensitivity	58,6
Specificity	89,8
Positive predictive value	78,4
Negative predictive value	77,4
Prevalence	38,7
Accuracy	77,7

THC		MS result	
		+	-
On-site result	+	58	16
DRAEGER	-	41	141

Table 11: Results for cocaine detection with Dräger.

DRAEGER –COCAINE	
N	256
True positives	82
True negatives	163
False positives	2
False negatives	9
Sensitivity	90,1
Specificity	98,8
Positive predictive value	97,6
Negative predictive value	94,8
Prevalence	35,5
Accuracy	95,7

COCAINE		MS result	
		+	-
On-site result	+	82	2
DRAEGER	-	9	163

Table 12: Results for methamphetamine detection with Dräger.

DRAEGER - METHAMPHETAMINE	
N	256
True positives	3
True negatives	244
False positives	2
False negatives	7
Sensitivity	30
Specificity	99,2
Positive predictive value	60
Negative predictive value	97,2
Prevalence	3,9
Accuracy	96,5

METHAMPHETAMINE		MS result	
		+	-
On-site result	+	3	2
DRAEGER	-	7	244

Table 13: Results for amphetamine detection with Dräger.

DRAEGER – AMPHETAMINE	
N	256
True positives	5
True negatives	217
False positives	31
False negatives	3
Sensitivity	62,5
Specificity	91,2
Positive predictive value	13,9
Negative predictive value	98,6
Prevalence	3,1
Accuracy	86,7

AMPHETAMINE		MS result	
		+	-
On-site result	+	5	31
DRAEGER	-	3	217

Table 14: Results for opiates detection with Dräger.

DRAEGER - OPIATES	
N	256
True positives	2
True negatives	247
False positives	0
False negatives	7
Sensitivity	22,2
Specificity	100
Positive predictive value	100
Negative predictive value	97,2
Prevalence	3,5
Accuracy	97,3

OPIATES		MS result	
		+	-
On-site	result	+	2
DRAEGER		-	7
			247

Descriptive statistics of the confirmation results

A copy of model for data collection form is attached as Appendix (A). Tables 5 to 9 shows the summary results related to each drug with the ORALAB device and tables 10 to 14 give the results with the DRÄGER device.

Each subject included in the study was asked to sign a consent form prior to participating and prior to collecting the oral fluid. A copy of the consent form is included as Appendix (B). This form was agreed by consensus en the Strasbourg meeting.

CONCLUSION

The Oralab device was not sensitive and accurate enough to detect amphetamines, but it really works very well with the others drugs, cannabis included. The main problem related to this device is that is not accepted by the police officers, because the interpretation is subjective. From our point of view it must be improved with the incorporation of an automatic reader. The Dräger device has shown problems of accuracy and sensitivity for cocaine and cannabis, the most frequently encountered drugs in our cases. Support from Dräger for any problems that developed over the period of use was excellent, and members of staff participated directly in the controls. Our participation in ROSITA I Project had positive consequences. The responsible authorities of Traffic Road in Spain are preparing laws on driving in the same way as other European countries. In fact is the first time that the General Directorate is trying to exchange the law in order to consent in same cases to take a blood sample, in order to establish that driving under influence of a drug, will be proven by the presence of this drug in the blood of the driver. But a important approach is the use of a onsite device to provide the agents with a presumptive evidence of recent drug use.

As in ROSITA I, the Traffic Police was very collaborative during all phases of study.

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General Traffic Directorate, Ministry of Interior.

All police officers that were involved in the controls. They worked many hours (always at daybreak) with great professionalism.

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ROSITA 2

PROTOCOLO PARA RECOGIDA DE MUESTRA INFORMACION DEL AGENTE DE TRÁFICO

Lugar: _____ Caso n° 3918 _____ ID Agente _____ Fecha/Hora _____

Razones por las que se efectúa el control:

- Conductor admite consumo Tipo de droga: _
- ☐ Existe parafernalia de drogas en el vehículo
- ☐ Nivel de alcohol étlico inconsistente con el deterioro.
- ☐ Presenta signos

TIPO DE DISPOSITIVO USADO: _____

Indicar Resultado Positivo (+) y Negativo (-) para cada droga.

	ANFETAM	COCAINA	THC	OPIACEOS	Fecha/hora
RESULTADOS					

Muestras: Saliva _____

- ☐ Rehusa proporcionar muestras.

.....

SOLO PARA USO DEL LABORATORIO

El laboratorio indicará Resultados Positivos (+), negativos (-) y niveles si es posible.

DROGA	CONFIRMACION DEL ANALITO	FLUIDO ORAL(ng/mL)		
		ORALAB	SPITTING	DRAEGER
ANFETAMINAS	ANFETAMINA			
	MDMA			
	Otros			
COCAINA	Cocaína			
	Benzoilecgonina			
	Otros			
THC	9-THC			
	THC-CCOOH			
OPIACEOS	Morfina			
	Codeína			
	MAM			



ROSITA 2

EVALUACIÓN DE DISPOSITIVOS PARA LA DETECCION DE DROGAS EN LA SALIVA.

A Ud. se le está preguntando si quiere participar en un estudio de investigación financiado por la Unión Europea (UE), para evaluar la seguridad y utilidad de nuevos dispositivos para detectar drogas de abuso en saliva. Para hacerlo Ud. debe de firmar este consentimiento y suministrar muestras de biológicas.

.....

CONSENTIMIENTO PARA PARTICIPAR EN EL PROYECTO DE INVESTIGACION

Entiendo que en el caso de suministrar muestras biológicas:

- Las muestras sólo serán usadas con propósitos de investigación
- Los resultados serán mantenidos confidencialmente
- Mi datos de identificación no se relacionarán con los resultados
- No hay riesgos conocidos en mi participación
- Los dispositivos de saliva están empaquetados higiénicamente, y la persona que recoge las muestras de saliva ha sido entrenada para recoger la muestra con absoluta seguridad.
- Los resultados del test no podrán ser usados como evidencia contra mi persona en un proceso penal o civil; sólo serán usadas con propósitos de investigación.
- Mi participación es completamente voluntaria
- No habrá consecuencias si declino participar en el estudio, pudiéndolo hacer en cualquier momento.

Sabiendo estas cuestiones firmo este consentimiento permitiendo la realización del estudio, y por la presente otorgo a los investigadores permiso para obtener las siguientes muestras biológicas:

Saliva_____Orina_____Sangre_____

Firma de la persona sujeta a investigación_____ **Fecha**_____

Firma del testigo_____ **Fecha**_____

ESTE PROYECTO SE ENCUENTRA FINANCIADO POR LA UNION EUROPEA BAJO LA COORDINACIÓN EN ESPAÑA DEL PROF. M.López-Rivadulla Lamas, Catedrático de Toxicología de la UNIVERSIDAD DE SANTIAGO DE COMPOSTELA, Y EN COLABORACIÓN CON EL SECTOR DE TRAFICO DE LA GUARDIA CIVIL DE GALICIA (Teniente Coronel J.L Ulla), así como la DIRECCIÓN PROVINCIAL DE TRAFICO (D.Gonzalo Ocampo).

Para cualquier aclaración acerca del proyecto, riesgos que se pueden correr, y/o sus derechos a participar, Ud. puede contactar con las siguientes personas:

Prof. Manuel López-Rivadulla Lamas.

Universidad de Santiago de Compostela. Instituto de Medicina Legal. Servicio de Toxicología Forense.

Telefono: 0034-981-582327

INTRODUCTION AND OVERVIEW OF ROSITA 2 U.S.A. OPERATIONS

Background: In February 2002 Dr. J. Michael Walsh, The Walsh Group (TWG) briefed the Director, of The White House Drug Policy Office, Office of National Drug Control Policy (ONDCP) regarding drugged driving as a public health policy issue. The briefing focused on various research implications and data for the conduct of an international study on drugged driving. During the briefing Dr. Walsh proposed a project to conduct a joint US/European international study to (1) assess the prevalence of illegal drug use among drivers failing a standard roadside sobriety test and (2) determine if it were feasible for police officers to detect drug use by drivers at the roadside through the use of state-of-the-art saliva drug testing devices. As a result of this briefing The Walsh Group in collaboration with Dr. Alain Verstraete [Ghent University of, Belgium] established a collaborative agreement to conduct a joint US/European international study to assess the prevalence of illegal drug use among drivers failing a standard field sobriety test and determine the feasibility of police use of saliva drug testing devices at the roadside to verify drug use by drivers. This project has become known as ROSITA 2.

Research Concept: Basic goals for this joint US/European international study were to conduct a to 1) assess the prevalence of illegal drug use among drivers failing a standard field sobriety test; and 2) determine the feasibility of police use of saliva drug testing devices at the roadside to verify drug use by drivers. The project would be conducted in major cities in the US and Western Europe by teams composed of scientists working in collaboration with local police. The intent of the project was to build on the success and the lessons learned in the original European ROSITA project that evaluated drug testing devices in eight European nations. The primary goal of study would be to evaluate **innovative saliva drug detection technology** for use by police either at the roadside or at a police station.

Principal Funding Partners: This project was a joint venture between:

- 1) The National Institute on Drug Abuse [NIDA]
National Institutes of Health, US Department of Health and Human Services
- 2) The National Highway Traffic Safety Administration [NHTSA]
US Department of Transportation
- 3) The Office of National Drug Control Policy
Executive Office of the President and
- 4) The European Commission's Directorate-General Transport and Energy [DG-TREN]

Methods: Standardized training of all research teams were conducted in the initial phases of the project to insure that the protocol was carried out uniformly across all study sites. In addition a core set of data variables [e.g. demographic, behavioral, and toxicological] was agreed to by all partners that would facilitate cross-site comparisons. To maximize the number of potential subjects, the study was carried out primarily on weekend nights between 11 p.m. and 6 a.m. and at locations known to produce DUI suspects. Standard police measures were used to identify drivers suspected to be under the influence of alcohol or drugs. Saliva specimens were collected from all DUI suspects who were tested for alcohol use [i.e. those failing a standard field sobriety test]. In cases where the saliva tests positive for illegal drugs, blood or urine were also collected to cross-validate the saliva test results. The goal was to collect a minimum of 500 saliva specimens at each study site [Total n of approximately 4000].

Prior to assigning devices to the research sites, a laboratory evaluation was conducted by The Walsh Group and the Center for Human Toxicology, University of Utah to determine whether they could meet specified criteria [e.g. a multi-analyte device, formulated to meet specified cutoff criteria, had to be able to give a result at the point of collection within minutes (either at the roadside or at a police station), suitable for use by police officer etc.]. Those devices meeting criteria were included in the evaluations. In the end three evaluations for twelve different devices were conducted using the same protocol (insert article references).

There were two separate evaluation periods lasting nine months each wherein different devices were evaluated. After the initial collection phase a meeting was convened for all the research teams to discuss problems encountered and make any mid-course modifications.

The first data collection phase started in October 2003 and lasted through June 2004. A progress meeting was held in Santiago de Compostela, Spain in December 2004 to review Phase I data and plans were established for the second part of the evaluation. Phase II continued through November 2005 for the EU countries and is to continue through July 2006 for the U.S. sites. The following chronology outlines the key events marking progress for the entire project:

Project Chronology

Date	Event
Jul 2003	Meeting Strasbourg France to establish protocol and project schedule
Aug 2003	Continue recruitment of US sites
Oct 2003	Initiation of Phase I data collection.
Jul 2004	End of Phase I data collection. Start Phase I data analysis
Nov 2004	Progress meeting Santiago de Compostela, Spain
Jan 2005	Initiation of Phase II data collection EU sites
Jan –May 2005	Training US sites for initiation of Phase II data collection
Aug 2005	Completion of Phase II data collection EU sites
May 2005 – Jul 2006	Phase II data collection for US sites
Dec 2006	Progress meeting Baltimore, MD.
Jan – Mar 2006	Prepare final report for EU sites. Interim report for US sites
Jul 2006	Completion of Phase II data collection for US sites
Dec 2006	Final report US sites

Outcomes -- The results of these evaluations could provide:

- Data regarding the accuracy/reliability of saliva tests for use at the roadside/police station
- Data regarding the practical and operational aspects of the use of on-site saliva tests
- Data to demonstrate that illegal drug use by drivers is a serious, growing problem
- Comparison data to estimate the global prevalence of driving under the influence of drugs other than alcohol [DUID]
- Data to establish the prevalence of illegal drugs being used in combination with alcohol
- Insights as to whether the current detection capabilities used by police at the roadside are acceptable

Research Leaders:

Dr. J. Michael Walsh and Dr. Alain Verstraete coordinated the US and EU research teams respectively.

The U.S. Partners: US research teams were identified in major metropolitan areas in the US who could meet specified conditions. Criteria for team selection was based on research reputation, the assurance of police collaboration, projection of sufficient volume of DUI suspects to achieve study goals, drug-testing analytical skills of the research team, and required budget. All teams were required to follow standardized protocols where as many variables as possible would be held constant and the project would run for a fixed duration.

Initially, teams were established at the following locations in the U.S.:

State	Police Department(s)	Coordinator/Lab
Florida	Hillsborough County Sheriff's Office	The Walsh Group/FDLE
	Manatee County Sheriff's Office*	The Walsh Group/FDLE
Washington	Washington State Police	Washington State Toxicology Lab
Utah	Salt Lake City PD	Center for Human Toxicology
Wisconsin	12 Police Jurisdictions	Wisconsin State Lab of Hygiene
Indiana	Indiana State Police*	State Department of Toxicology

* Dropped from participation in project

A detailed summary of the project operations and results are included in the following sections.

- A. Florida Results
- B. Washington Results
- C. Utah Results
- D. Wisconsin Results

Chapter 9

Evaluation of Roadside Oral Fluid Drug Testing Equipment/Devices

Site: Florida – Hillsborough County Sheriff's Office, Tampa Florida USA

Coordinator: Dr. J. Michael Walsh – U.S. Coordinator

Partners: Contribution of Hillsborough County Sheriff's Office, Tampa, Florida, Major Eugene Stokes, (2003-2004), Major Paul Davis, (2005-2006), Site Coordinator, Sgt. Ronald Harrison (2005-2006), Sgt. Laura Regan, (2003-2004), and Karen Hernandez.

Authors: Leo Cangianelli, J. Michael Walsh, Ph.D.

Date: January 31, 2006

Abstract

This study was a collaborative US/EU international effort to assess the prevalence of illegal drug use among drivers failing a standard field sobriety test [i.e. DUI suspects] and to evaluate the effectiveness of oral fluid drug detection technology to detect drug abuse by drivers at the roadside. The project was conducted in major cities in the US and Western Europe by teams composed of scientists working in collaboration with local police. This paper presents analysis of data from one of the U.S. sites that participated in the larger study. The information was collected by The Walsh Group in partnership with the Hillsborough County Sheriff's Office (HCSO) Tampa, Florida. Two oral fluid drug testing devices were evaluated by HCSO over two nine month collection periods with 140 specimens collected/tested. Unfortunately, the results of these analyses are incomplete at this time since the U.S. phase II data collection is ongoing. The final results of phase I and phase II will be the subject of a final report published by all U.S. sites later in 2006.

Introduction

The Walsh Group in collaboration with Dr. Alain Verstraete [Ghent University, Belgium] established a collaborative agreement to conduct a joint US/European international study to assess the prevalence of illegal drug use among drivers failing a standard field sobriety test and determine the feasibility of police use of saliva drug testing devices at the roadside to verify drug use by drivers. This project was conducted in major cities in the US and Western Europe by teams composed of scientists working in collaboration with local police. Intent of the project was to build on the success and the lessons learned in the original European ROSITA project that evaluated drug testing devices in eight European nations. For this project, four teams were identified in the United States located in Washington State, Salt Lake City, Utah, Wisconsin State and Florida. This section reports on the results of the study from the Hillsborough County Sheriff's Office located near Tampa, Florida, United States of America.

The Florida DUID law states that a person is guilty of the offense of driving under the influence and is subject to punishment as provided in subsection (2) if the person is driving or in actual physical control of a vehicle within the state and: (a) The person is under the influence of...any chemical substance...or any controlled substance..., when affected to the extent that the person's normal faculties are impaired. There is implied consent for testing of urine only. Special circumstances exist requiring a driver to provide a blood sample for testing when he/she has been involved in a crash resulting in death or serious bodily injury.

The Florida law is silent on the collection of saliva as part of a DUI evaluation. As a result, prior to initiating data collection in Florida, The Walsh Group asked the Florida State Attorney General for and received an exception to allow police officers to collect oral fluid pursuant to the study protocol.

Initially, two sites were identified in Florida, Hillsborough County Sheriff's Office near Tampa and Manatee County Sheriff's Office in Bradenton, FL. Manatee County was unable to obtain the requisite number of specimens (250 each for Phase I and Phase II) and was dropped from the evaluation. Later in 2004, Miami was identified as a replacement site for Manatee County, but they too had to be dropped from participation due to their inability to obtain Human Subjects clearance and developing problems with their laboratory contract.

Phase I

Methods

Overview:

During phase I subjects were recruited by police officers after a DUI stop, and, if suspected of being under the influence of drugs, were taken to the central breath testing facility (CBT) where consent was obtained and further testing under the protocol. All officers were trained at the beginning of the collection period on the administrative procedures to be used (i.e. obtaining consent of the subject and data collection/recording) and on the operation of the oral fluid drug testing device. This training was repeated on two other occasions during the phase I collection period to sustain officer interest and refresh them regarding the project requirements. The goal was to collect 250 specimens over the nine month period (approximately 6-7 specimens each week).

Specimens collected: HCSO collected two oral fluid specimens and urine. One oral fluid specimen was collected using the assigned experimental device. The protocol for the assigned device required the drug testing device to be inserted in the donor's mouth and manipulated to collect the required amount of oral fluid. The second specimen was collected in the provided Intercept collection pad and forwarded in the transport vial to the University of Utah's Center for Human Toxicology (CHT) for laboratory confirmation. (See Appendix A for procedures involving collection of the Intercept specimen). A urine specimen was collected as required by Florida law for a DUID offense and was analyzed by the Florida Department of Law Enforcement (FDLE) laboratory. The urine results were forwarded to The Walsh Group for inclusion in the final analysis.

Device Assigned: For Phase I, HCSO was assigned the Branam "Oratect" drug test that had been evaluated in the second round of lab tests conducted by The Walsh Group and The Center For Human Toxicology. (Journal of Analytical Toxicology, Vol. 29, May/June 2005). This device is described in the following illustration.



Matrix: Saliva
Available for: Methamphetamine, Amphetamine, Opiates, Cocaine, Cannabinoids, and Phencyclidine
Number of parameters per device: 6
Not FDA approved
Storage conditions: Room temperature
Manipulations to obtain a result:
1) Collect a saliva sample by placing device inside the donor mouth on the inner cheek. The collection pad should touch the donor's cheek.
2) After 10 seconds move the device to the bottom of the mouth under the tongue. Leave the device under the tongue until the colored lines appear in the viewing windows and remove device from the mouth.
3) Read the results within 9 to 11 minutes after removing the device. Do not read results after 11 minutes. [N.B. The intensity of the colored line may change if the results are read after 11 minutes.]
Interpretation of the result: Visual
Save specimen for confirmation: Yes (Wick can be separated from the device and sent to lab)
User friendliness: Good

Drugs to be tested: The HCSO evaluation relied primarily on the results of the laboratory saliva (Intercept) tests results and urine results obtained from the Florida Department of Law Enforcement (FDLE) Laboratory to validate the Branan "Oratect" rapid oral fluid drug testing device. The primary reference was the laboratory saliva (Intercept) test results from CHT. Each specimen collected was tested for a core set of drugs as follows:

Saliva rapid Test: The Oratect device tested oral fluid for cannabis, cocaine, amphetamines, methamphetamines, and opiates.

<u>Branan test profile and cutoffs</u>		Opiates	20 ng/mL
Amp	50 ng/mL	Cocaine	20 ng/mL
Meth	50 ng/mL	THC	100 ng/mL
		THC-COOH	20 ng/mL

Laboratory saliva Test (Intercept): CHT screened the laboratory saliva (Intercept) specimen for cannabis, cocaine, amphetamines, methamphetamines, opiates, and benzodiazepines. A confirmation test using GC/MS or LC/MS was conducted whenever there was inconsistency between the saliva rapid screen (Oratect) and the saliva lab (Intercept) screen.

CHT Oral Fluid laboratory screening test profile

Amp/meth	40 ng/mL	Cocaine	5 ng/mL
Benzodiazepines	2 ng/mL	Opiates	10 ng/mL
THC	2 ng/mL		

Urine laboratory Test: Cannabis, cocaine, amphetamines, methamphetamines, opiates, and benzodiazepines. For Phase I, the FDLE laboratory tests were screening tests. Screened positives were confirmed using GC/MS. The urine results were used for information purposes only and in the final analysis.

FDLE Urine laboratory test profile

Amphetamines	500 ng/mL	Opiates	200 ng/mL
Benzodiazepines	100 ng/mL	Cocaine metabolites	300 ng/mL
THC	50 ng/mL	Methadone	200 ng/mL

Barbiturates 300 ng/mL

Inclusion Criteria: Under the protocol standard police measures were used to identify drivers suspected to be under the influence of alcohol or drugs. A driver was eligible for inclusion in the evaluation if he/she was stopped for any reason and the police officer has reasonable suspicion that the driver was operating the vehicle after using, or while under the influence of drugs.

Criteria used by police officers as reasonable suspicion of drug use were:

- The driver's appearance, behavior, or odor indicate drug use
- The driver failed the SFST or equivalent test; or
- The driver had illicit drugs or drug paraphernalia on his/her person or in the vehicle; or
- The driver admitted to using drugs; or
- The police officer believed that the BAC level was inconsistent with the level of observed impairment

Procedures: For drivers who met the above criteria, police officers took the subject to a Central Breath Testing facility (CBT) in the Orient Jail where the officer interviewed the subject, obtained his/her consent to participate, and collected oral fluid and urine specimens. This was accomplished after all evidential tests on the subject and paper work for the arrest were completed.

1. One oral fluid specimen was tested by the police officer using the Branan "Oratect" on-site drug testing device and a second oral fluid specimen was collected using the "Intercept" saliva collector. The results of the "Oratect" device were read by the police officer and recorded on the data sheet. The second "Intercept" specimen was annotated with the case number on the Intercept saliva transport vial and placed in a plastic envelope along with the data and consent sheets. This completed subject package was retained for final review by the site coordinator who aggregated and sent the Intercept vials to CHT for further testing. The data sheets and consent forms were forwarded to The Walsh Group for data entry and retention.
2. Urine specimens were handled in accordance with Florida law and sent to the FDLE laboratory for testing. After urine test results were received from the FDLE laboratory they were also forwarded to The Walsh Group for data entry.

Data Collection Form: All data required for the project was written on the Data Collection Form as described below. A copy is attached as Appendix (B) at the end of this section.

Case No. – A unique case number generally same as used for the arrest citation.

Officer ID – Officer's badge number or other officer identification.

Date of Stop: Month/day/year)

Time of Stop: Time of the stop (i.e. 9:45 pm).

Site Location – Preprinted on the form for each center.

Reason for Drug Test – Checked box indicating the reason for the drug test.

Reported BAC Test Results – Reported BAC level and time the BAC test was conducted.

Oral Fluid Drug Test Device – The name of the assigned saliva rapid drug-testing device

Officer's Test Results – Results indicating "+" or "-" for each drug tested.

Specimen Collection Time – Time each of the specimen(s) were taken.

Consent Form: A copy of the standard U.S. site consent form is included as Appendix (C). Each subject included in the study was asked to sign a consent form prior to participating and

prior to collecting the specimen(s). Normally, this was completed after all evidentiary specimens and forms for the arrest were concluded by the police officer.

Results Summary

Subjects Summary Phase I	Phase I
Device Evaluated	(Branan Oratect)
Number of Stops and subjects solicited	35*
Number of refusals	10*
Number of specimens collected/tested	25
Number of device failures	13
Number of OF specimens collected with complete data	12
Number of urine specimens collected	25

*These numbers are approximate as record of stops and refusals were incomplete

Device Evaluation Summary Phase I (Device: Branana – Oratect)

	Total	THC	Coc	Meth	Amp	Opi
Total assays conducted “n”	60	12	12	12	12	12
True positives	1	0	1	0	0	0
True negatives	52	10	7	12	12	11
False positives	1	1	0	0	0	0
False negatives	6	1	4	0	0	1
Sensitivity	14.3%	0.0%	20.0%	NA	NA	0.0%
Specificity	98.1%	90.9%	100.0%	100.0%	100.0%	100.0%
Positive predictive value	50.0%	0.0%	100.0%	NA	NA	NA
Negative predictive value	89.7%	90.9%	63.6%	100.0%	100.0%	91.7%
Prevalence	NA	8.3%	41.7%	0.0%	0.0%	8.3%
Accuracy	88.3%	83.3%	66.7%	100.0%	100.0%	91.7%

Discussion

Data collection throughout phase I was plagued with a number of problems and difficulties. Officers were not asking all potential subjects to participate and there were a large number of refusals on the part of the subjects. Some of the refusals resulted from a communication problem as there were many non-English speaking arrestees that did not clearly understand the purpose of the project and police officers were ineffective in communicating the requirements for inclusion. Additionally the Branana “Oratect” device failed to run or showed no result in far too many cases which discouraged police officers.

Many efforts were made to overcome these problems.

- A log was maintained documenting each officer’s attempts to obtain a specimen when drug use was suspected. This was started late in the phase I collection period.
- Two special re-training sessions were conducted to boost participation and ensure that officers were fully aware of their responsibilities.
- A special consent form was formulated in Spanish and the dialogue asking for consent was also translated into Spanish
- As often as possible, Spanish speaking police officers were assigned to the saturation patrols
- Saturation patrols were also increased in an effort to improve recruitment of subjects

- The device manufacturer modified the device to make it run faster and deal with the issue of dry mouth

Conclusions

Unfortunately, despite all these actions, only 35 specimens were collected and of these only 23 were complete (these are approximate numbers as complete records were not kept). In addition, the changes made by the device manufacturer failed to improve the device and created other problems in the device operation. As a result, the Oratect device was removed from use by HCSO in October 2004 and a replacement device was sought for Phase II operations. On the other hand many lessons were learned during phase I that made the phase II recruitment of subjects easier, improved the collection and handling procedures, and has also resulted in greatly improved numbers of specimens collected.

Phase II

Methods

Overview: During phase II, HCSO changed some of the procedures to increase subject recruitment for the study. One element that helped to improve the recruitment of subjects was the addition of a new Blood Alcohol Mobile Testing Unit (Batmobile) that was deployed by HCSO at the beginning of phase II operations. Also, at the outset of phase II, a research technician was hired to ride along with the police officers in the new Batmobile; and, when the Batmobile was not in use, to be positioned at the CBT during peak hours of most week-ends. The technician's responsibilities were to approach, interview and obtain consent from participating subjects; collect and test specimens; complete data sheets; ship completed subject packs to The Walsh Group; ship Intercept specimens to CHT for further testing; and assist the site coordinator as may otherwise be required. This new procedure worked well in Salt Lake City and has become a model for subject recruitment for other U.S. sites. As of this writing, HCSO has collected and tested 117 specimens, more than four times that of Phase I and collections will continue through June 2006.

Device Assigned: For Phase II, HCSO was assigned the Draeger "UpLink" device which had been evaluated in the second round of lab tests conducted by The Walsh Group and The Center For Human Toxicology. (Journal of Analytical Toxicology, Vol. 29, May/June 2005). This device is described in the following illustration.



Matrix: Saliva, [Collection kit includes: collection device, buffer cartridge, and test cassette]

Tests Available: Methamphetamine, Amphetamines, Opiates, Cocaine, marijuana, and PCP

Number of parameters per device: Six

Not FDA approved

Storage conditions: Room temperature

Manipulations to obtain a result:

Collect a saliva sample with the swab collection sponge.

- 1) Insert the buffer cartridge into the lower groove of the test cassette by aligning lower tabs on the cartridge with notches on the cassette. Rock cap and remove.
- 2) Insert the sample collection device into the buffer cartridge and press down on the sample collection device (notice that the sample collection device clicks slightly into place and a cracking sound can be heard when it is pushed down firmly).
- 3) Remove the handle of the sample collection device by means of a quarter turn anticlockwise.
- 4) Close buffer cartridge. Allow 4 minutes of incubation time.
- 5) Turn the buffer cartridge clockwise until the upper groove is positioned above the groove of the test cassette. Push down the buffer cartridge firmly until the cartridge wings touch the edge of the cassette. Allow the fluid to develop for 8 minutes.
- 6) After 8 minutes enter sample ID in the analyzer and insert the cassette into the analyzer.
- 7) Analyzer will indicate the door is closed and read cycle has begun. Analyzer will report results in 4 minutes.

Interpretation of the result: Digital read-out

Save specimen for confirmation: Possible

User friendliness: Fair

Specimens collected: In phase II HCSO also collected two oral fluid specimens and urine. One oral fluid specimen was collected using the “UpLink” collection device and the second collected in the Intercept collection device and forwarded in the transport vial to CHT for laboratory screening and confirmation. The urine specimen was collected as required by Florida law for a DUID offense and was analyzed by the FDLE laboratory. The urine results were forwarded to The Walsh Group for inclusion in the final analysis.

Drugs tested: As in phase I HCSO relied primarily on the results of the saliva laboratory (Intercept) tests and urine results obtained from the Florida Department of Law Enforcement (FDLE) Laboratory to validate the rapid oral fluid drug testing devices. The primary reference was the saliva laboratory (Intercept) test results. Each specimen collected was tested for a core set of drugs as follows:

Saliva rapid Test: The “UpLink” device tested oral fluid for amphetamine, cocaine, methamphetamine, opiates, and THC.

Draeger Uplink on site device profile

Meth.	25 ng/mL	THC	25 ng/mL
Amp.	25 ng/mL	Opiates	20 ng/mL
Cocaine	200 ng/mL		

Saliva laboratory Test (Intercept): CHT screened the saliva laboratory (Intercept) specimen for cannabis, cocaine, amphetamines, methamphetamines, opiates, and benzodiazepines. As in phase I, a confirmation test using GC/MS or LC/MS was conducted whenever there was inconsistency between the saliva rapid screen and the saliva lab screen. The panel was the same as used in phase I.

Urine laboratory Test: Urine was tested for cannabis, cocaine, amphetamines, methamphetamines, opiates, and benzodiazepines by the FDLE lab using the same procedures and panel as for Phase I.

Inclusion Criteria: Under the protocol standard police measures were used to identify drivers suspected to be under the influence of alcohol or drugs. Police officers continued to attempt to determine if the driver may have been under the influence of or had recently used drugs. However, in phase II all drivers were eligible for inclusion in the evaluation if he/she was stopped for any reason and the subject volunteered to participate in the study.

Procedures: For drivers who met the inclusion criteria for phase II, the technician recruited, instructed and obtained consent from the subject collecting oral fluid and urine specimens either in the Batmobile or at the CBT. This was accomplished after all evidential tests on the subject and paper work for the arrest were completed.

1. One oral fluid specimen was tested by the research technician using the “UpLink” on-site drug testing device and a second oral fluid specimen was collected using the “Intercept” saliva collector. The results of the “UpLink” device were read from the printout by the technician/police officer and recorded on the data sheet. The second “Intercept” specimen was annotated with the case number on the Intercept transport vial and placed in a plastic envelope along with the data and consent sheets. This completed subject package was retained for final review by the technician and/or site coordinator who aggregated and sent the Intercept vials to CHT for further testing. The data sheets and consent forms were forwarded to The Walsh Group for data entry and retention.
2. Urine specimens were handled in accordance with normal site procedures and sent to the FDLE laboratory for testing. After urine results were received from the FDLE laboratory they were also forwarded to The Walsh Group for data entry.

Data Collection Form: All data required for the project was written on the Data Collection Form as described in phase I.

Consent Form: Procedures were same as used in phase I.

Subjects Summary Phase II

	Phase II
Device Evaluated	(Draeger UpLink)
Number of Stops and subjects solicited	Unknown*
Number of refusals	Unknown*
Number of specimens collected/tested	122
Number of device failures	5
Number of OF specimens collected with complete data	117
Number of urine specimens collected	18

* Data not recorded by police

Device Evaluation Summary Phase II – Interim Data (Device: Draeger – UpLink)

	Total	THC	Coc	Meth	Amp	Opi
Total assays conducted “n”	585	117	117	116	117	117
True positives	22	9	10	1	2	0
True negatives	519	76	102	115	113	113
False positives	20	16	1	0	2	0
False negatives	24	16	4	0	0	4
Sensitivity	47.8%	36.0%	71.4%	100.0%	100.0%	0.0%
Specificity	96.3%	82.6%	99.0%	100.0%	98.3%	100.0%
Positive predictive value	52.4%	36.0%	90.9%	100.0%	50.0%	NA
Negative predictive value	95.6%	82.6%	96.2%	100.0%	100.0%	96.6%
Prevalence	NA	21.4%	12.0%	0.9%	1.7%	3.4%
Accuracy	92.5%	72.6%	95.7%	100.0%	98.3%	96.6%

Discussion of Results

As a result of the measures taken to increase subject participation, sufficient subjects were recruited in the initial stages of phase II to warrant continuing the project through June 2006. Unfortunately, in the interim, the Draeger Corporation made a business decision to halt production of the “UpLink” device and will no longer support it. HCSO will continue to use up the UpLink cassettes until they expire and a replacement device will be placed into service there for the remainder of the evaluation.

Conclusions

The Branam Oratect I used in phase I was not suitable for roadside testing for two primary reasons:

- The device was not sensitive and accurate enough to detect the two primary drugs of abuse, i.e. marijuana and cocaine
- The device suffered a large number of failures (they failed to run to completion) that discouraged the police from using them.

Since the time that phase I was conducted Branam has replaced the device the Oratect II with supposedly superior characteristics and specifications.

The instrumented “UpLink” device evaluated in phase II worked well in a stable setting such as the Batmobile or the centralized BAT center where sufficient room and power were available. The device however, had some limitations:

- Initial results indicate that the sensitivity for detecting THC is still not as good as the police would like to see it.
 - Overall there were 25 specimens positive for THC (nine were true positives and there were 16 false negatives)
 - The overall prevalence for THC using the UpLink was 25 of 117 tested or 22%.
- Reliability of the instrument was satisfactory and support from Draeger for any problems that developed over the six month period of use was excellent.
- All of the information appears to be moot in view of the withdrawal of the instrument from the drug testing marketplace.

Overall, in both phase I and phase II, the laboratory saliva collected using the Intercept pads worked well for laboratory testing and were more accurate than the on-site tests.

APPENDIX A

Intercept Oral Fluid Collection Procedures



Step 1

Inspect the expiration date on the Intercept® collection device packaging and open the outer packaging containing the collection pad and the specimen vial. Place the specimen vial on a flat surface in view of the donor. Instruct the donor to remove the handle and remove the collection device from the packaging sleeve.



Step 2

Instruct the donor to place the collection pad between the lower cheek and gums and gently rub the pad back and forth along the gum line until the pad is moist.



Step 3

Once moist, leave collection pad between cheek and gums for a full two (2) minutes.



Step 4*

After 2 minutes, have the donor open the specimen vial in an upright position, with the tip pointed downward, by gently rocking the cap back and forth to avoid spilling the contents. Instruct the donor to push the collection pad into the specimen vial as far as it will go.



Step 5*

Instruct the donor to snap the collection wand at the scored line against the side of the vial. Instruct the donor to not tilt the vial or spill the fluid inside.



Step 6*

Instruct the donor to place the cap onto the vial until it snaps, ensuring a secure fit.



Step 7*

Instruct donor to place tamper evident seal across the top of the specimen vial and down the sides, date and initial.

*At some sites Steps 4 through 7 were accomplished by the collector.

APPENDIX B

CASE NO: _____

OFFICER ID: _____

ROSITA2 DATA COLLECTION FORM

POLICE OFFICER INFORMATION

Date of Stop: _____

Time of Stop: _____

Site Location: HCSO

Reason For Drug Test:

Reported BAC Test Results:

☐ Unusual appearance, behavior, or odor indicating drug use☐ Failed SFST and BAC level < 0.08☐ Had drug / drug paraphernalia in vehicle☐ BAC level inconsistent with impairment☐ Driver admits to using drugs. Which drug(s)? _____

BAC Level	Time

Saliva Rapid Drug Test Device: **Uplink**

Indicate Positive Result with (+) and Negative with (-) for each drug

	AMP	METH	COC	THC	OPI
Test Results					

Specimen Collection Date/Time: Oral Fluid: _____ Urine: _____
Collection Time Collection TimeOfficers Comments: _____

FOR LABORATORY USE ONLY

Lab Accession Number: _____

Indicate Positive with (+) and Negative with (-) and quantitative concentration (ng/mL)

Drug	Confirmation Analyte	Oral Fluid		Urine	
		Screen	Confirm	Screen	Confirm
AMP	AMP / METH	(+) or (-)	ng/mL	(+) or (-)	ng/mL
	Amphetamine				
	Methamphetamine				
METH	MDMA (Ecstasy)				
	MDEA				
	MDA				
COC	COC				
	Cocaine				
	Benzoylcegonine				
THC	THC				
	9THC (parent)				
	THCCOOH (metab.)				
OPI	OPI				
	Morphine				
	Codeine				
	Hydromorphone				
	Oxycodone				
BDP	BDP				
	Diazepam				
	Oxazepam				
PCP	PCP				
ETOH	(If Determined)				

Comments: _____

APPENDIX C

TITLE OF STUDY: ROSITA 2 – Evaluation of Oral Fluid (Saliva) Drug Detection Devices

You are being asked to be in a research study to test new drug detection devices that use saliva. To be in the study, all you need to do is sign this consent form and provide saliva specimens.

CONSENT TO BE IN THE RESEARCH STUDY

If I choose to consent and provide saliva specimens:

- ✓ All information that might tie my name to the results will be removed after all testing is completed. Therefore, after that is done my name cannot be connected to the results
- ✓ The specimens will be used for research purposes only
- ✓ The results will be kept completely confidential
- ✓ There are no known risks to my being in the study
- ✓ The saliva collection devices are sterile and the person collecting the saliva has been trained to do so in a manner that is safe
- ✓ The results of my saliva tests cannot be used against me in court
- ✓ My being in the study is completely voluntary
- ✓ Nothing will happen to me if I choose not to be in this study
- ✓ I may stop and quit the study at any time

Knowing all this, I agree to be in this study and give you permission to collect saliva specimens from me for research purposes.

Research Subject – Sign Here:

Witness – Sign Here:

This project is being funded by the Office of National Drug Control Policy (ONDCP), The National Institute on Drug Abuse (NIDA), and The National Highway Traffic Safety Administration (NHTSA) under the direction of The Walsh Group, P.A. and in cooperation with this police agency.

If you have any questions about the research project, the risks involved, and/or your rights as a participant, you may contact us at the following number / address:

J. Michael Walsh, Ph.D., Principal Investigator
6701 Democracy Blvd., Suite 300
Bethesda, MD 20817
Telephone: 1-800-985-1225

Original – To Subject

Chapter 10 Evaluation of Roadside Oral Fluid Drug Testing Devices

Site: Salt Lake City, Utah USA

Coordinator: Dennis J. Crouch

Partners: The Salt Lake City Police Department, Salt lake City, UT. Sergeant LaMar Ewell (primary contact), Chief Rick Dinse, Assistant Chief Ken Pearce, Assistant Chief Don Llewellyn (retired) and Captain Carroll Mays. The Utah Public Health Forensic Toxicology Laboratory - Terry Lamoreaux, then Director.

Authors: «GreetingLine» and Oscar Quintela

Date: February 3, 2006

Abstract

This study was a collaborative US/EU international effort to assess the prevalence of illegal drug use among drivers failing a standard field sobriety test [i.e. DUI suspects] and to evaluate the effectiveness of oral fluids drug detection technology to detect drug use by drivers at the roadside. The project was conducted in major cities in the US and Western Europe by teams of scientists working in collaboration with the local police. This paper presents analysis of data from one of the U.S. sites (Salt Lake City) that participated in the larger study. The information was collected by The Walsh Group in partnership with the Salt Lake City Police Department (SLCPD) Salt Lake City, Utah and the Center for Human Toxicology at the University of Utah, Salt Lake City, UT. The oral fluids drug testing device (Drugwipe, Securetec) was evaluated during two nine-month collection periods with 249 specimens collected and 210 fully tested to date. The results presented here are preliminary at this time because the U.S. phase II data collection is ongoing. The combined results of phase I and phase II will be the subject of a final report published by all U.S. sites later in 2006.

Introduction

The Walsh Group in collaboration with Dr. Alain Verstraete [Ghent University, Belgium] established a collaborative agreement to conduct a joint US/European international study to assess the prevalence of illegal drug use among drivers failing a standard field sobriety test and determine the feasibility of police use of oral fluids (OF) drug testing devices at the roadside to verify drug use by drivers. This project was conducted in major cities in the US and Western Europe by teams of scientists working in collaboration with local police. The intent of the project was to build on the success and the lessons learned in the original European ROSITA project that evaluated drug testing devices in eight European nations. For the current ROSITA study, four teams were identified in the United States they were located Salt Lake City, Utah; Washington State; Wisconsin State and Florida. This section reports study results from Salt Lake City, UT.

The Utah DUID law states that a person may not operate or be in actual physical control of a vehicle within the state if the person...is under the influence of ...any drug...to a

degree that renders the person incapable of safely operating a vehicle. Also, a person may not operate or be in actual physical control of a motor vehicle within the state if the person has any measurable controlled substance or metabolite of a controlled substance in the person's body.

Phase I

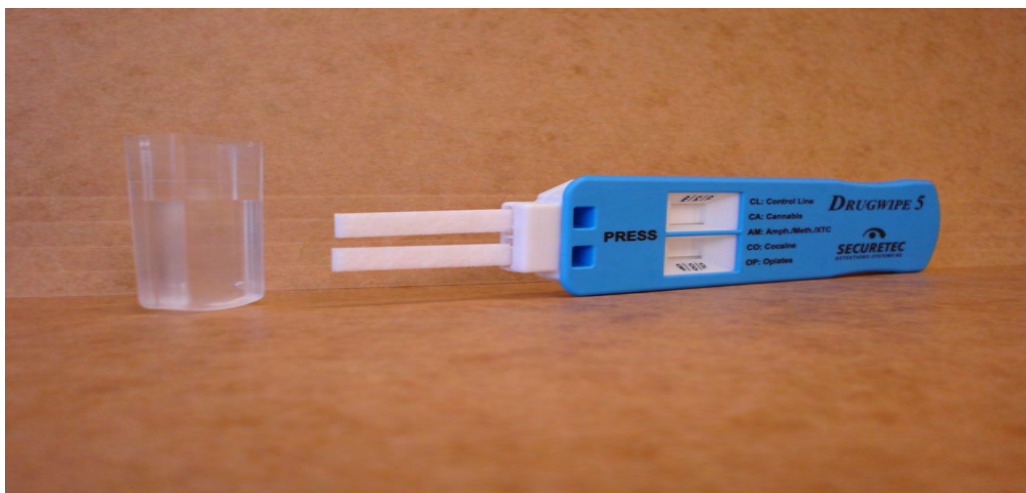
Methods

Overview:

All officers were trained at the beginning of the study period (December, 2003) on the administrative procedures to be used (i.e. consenting of the subject and data collection/recording) and on the use of the OF drug testing and collection devices. However, during phase I, SLC was in the unique situation of having an on-call phlebotomy technician who was summoned to the site of each DUI arrest for a blood collection. Therefore, that technician was used to recruit the subjects. It was hypothesized that he would be perceived to be more scientific and, perhaps, less involved in the arrest and legal consequences than a uniformed officer. The phase 1 goal was to recruit 250 subjects and collect 250 specimens during a nine-month period (approximately 6-7 specimens each week).

Specimens collected: SLC collected two OF specimens and a blood specimen from each participant. One OF specimen was collected and tested using the Drugwipe device. The second OF specimen was collected with the Intercept and forwarded in its transport vial to the University of Utah's Center for Human Toxicology (CHT) for laboratory analysis. (See Appendix A for OF collection using the Intercept). The blood specimen was collected in accordance with the Utah law for DUID offenses and was analyzed by the forensic toxicology laboratory at the Utah Department of Health. The blood results were forwarded to The Walsh Group for data compilation.

Device Assigned: For phase I, SLC was assigned the Securetec "Drugwipe" device that had been evaluated in the laboratory by The Walsh Group and CHT. (Journal of Analytical Toxicology, Vol. 29, May/June 2005). The Drugwipe 5 device is shown in the illustration below. The current model is quite similar except that it has 2 collection strips.



Device use:

Matrix: Oral fluids, sweat

Available for: Amphetamine and Methamphetamine, Opiates, Cocaine and Cannabinoids

Not FDA approved

Storage conditions: Room temperature

Manipulations to obtain a result:

- 1) Disconnect wiping section from the device
- 2) Wipe the surface of the tongue or the body for approximately 10 sec
- 3) Reassemble the device and dip the absorbent pads into water for a 10 count
- 4) Read the result after approximately 2 min

Interpretation of the result: Visual

Save specimen for Confirmation: Possible

User friendliness: Good

User friendliness: Good

Drugs tested: The SLC evaluation compared the laboratory OF (Intercept) immunoassay and blood results obtained from Department of Health laboratory to those obtained from the Drugwipe using the Intercept results as the reference.

Each specimen collected was tested as follows:

Oral Fluids rapid Test: The Drugwipe tested OF for the presence of cannabis, cocaine, amphetamines, methamphetamines and opiates.

Drugwipe test profile and cutoff concentrations

Amp	100 ng/mL	Cocaine	50 ng/mL
Meth	100 ng/mL	THC	30 ng/mL
Opiates	20 ng/mL	THC-COOH	3 ng/mL

Laboratory Oral Fluids Test (Intercept): CHT screened the Intercept specimen for the presence of cannabis, cocaine, amphetamines, methamphetamines, opiates and benzodiazepines. A confirmation using GC/MS or LC/MS/MS was conducted whenever there was any inconsistency between the OF rapid screen and the OF laboratory and blood results.

CHT Oral Fluids laboratory screening test profile (ng/mL buffer)

Amp/meth	50/25 ng/mL	Cocaine	8 ng/mL
Benzodiazepines	2 ng/mL	Opiates	20 ng/mL
THC	2 ng/mL		

Laboratory Blood Test: Laboratory tested analytes included cannabis, cocaine, amphetamines, methamphetamines, opiates and benzodiazepines. The blood cutoff concentrations for the laboratory screening and confirmation tests are shown below. Screened positive samples were confirmed using GC/MS. The blood results were used for information purposes in accessing discrepancies between the on site and laboratory OF tests.

Laboratory testing profile (screen/confirmation)

Amphetamines	50/50 ng/mL	Benzodiazepines	50/varied by drug
--------------	-------------	-----------------	-------------------

THC	10/1 ng/mL	Cocaine metabolites	50/50 ng/mL
Opiates	50/50 ng/mL		

Inclusion Criteria: In compliance with the protocol, standard police measures were used to identify drivers suspected of being under the influence of alcohol or drugs. A driver was eligible for inclusion in the study if he/she was detained for any reason and the police officer had reasonable suspicion that the driver was operating the vehicle after using, or while under the influence of drugs.

Criteria used by police officers as reasonable suspicion of drug use were:

- The driver's appearance, behavior or odor indicate drug use
- The driver failed the SFST or equivalent test; or
- The driver had illicit drugs or drug paraphernalia on his/her person or in the vehicle; or
- The driver admitted to using drugs; or
- The police officer believed that the BAC was inconsistent with the degree of observed impairment

Procedures: For drivers who met the above criteria and police officers suspected impairment, the phlebotomist was summoned to collect a blood specimen and recruit the subjects into the study. Informed consent was obtained and the OF samples collected. The results of the Drugwipe were interpreted by the technician and recorded on the data sheet. The second OF, or Intercept, specimen was placed in its transport vial, labeled with the case number and secured in a plastic envelope along with the data collection and consent forms. The site coordinator reviewed the completed case materials and the Intercept specimens were forwarded to CHT for analyses. The data sheets and consent forms were forwarded to The Walsh Group for data entry and retention. Samples were identifiable at CHT by study number only.

Blood specimens were handled in accordance with normal SLCDP procedures and sent to the Utah Health Department laboratory for analysis. After the analyses were completed, the results were forwarded to The Walsh Group for data entry.

Data Collection Form: All data required for the project was recorded on a Data Collection Form as described below. A copy is attached as Appendix (B) at the end of this section.

Case No. – A unique case number generally same as used for the arrest citation.

Officer ID – Officer's badge number or other officer identification.

Date of Stop: Month/day/year)

Time of Stop: Time of the stop (i.e. 9:45 pm).

Site Location – Preprinted on the form for each center.

Reason for Drug Test – Checked box indicating the reason for the drug test.

Reported BAC Test Results – Reported BAC level and time the BAC test was conducted.

Oral Fluids Drug Test Device – The name of the assigned OF rapid drug-testing device

Officer's Test Results – Results indicating "+" or "-" for each drug tested.

Specimen Collection Time – Time each of the specimen(s) were taken.

Consent Form: A copy of the IRB approved informed consent form used in SLC is shown in Appendix (C). Only after all evidentiary specimens were collected and all forms for the arrest

were completed by the police officer were subjects asked to participate in the study. Before subjects were enrolled they were required to provide informed consent and only then were OF specimens collected for the study.

Results Summary

Subjects Summary Phase I	Phase I
Device Evaluated	(Securetec Drugwipe)
Number of Stops and subjects solicited	Not recorded*
Number of refusals	Not recorded*
Number of specimens collected/tested	41
Number of device failures	1
Number of OF specimens collected with complete data	40
Number of blood specimens collected	41

*No record of stops and refusals was maintained

Device Evaluation Summary Phase I (Preliminary*) (Device: Securetec – Drugwipe)

	Total	THC	Coc	Meth /Amp**	Opi
Total assays conducted “n”	160	40	40	40	40
True positives	27	14	6	2	5
True negatives	99	9	27	31	32
False positives	31	16	5	9	1
False negatives	3	1	2	0	0
Sensitivity	90.0%	93.3%	75.0%	100.0%	100%
Specificity	76.2%	36.0%	84.4%	77.5%	97.4%
Positive predictive value	46.6%	46.7%	54.5%	18.2%	66.7%
Negative predictive value	97.1%	90.0%	93.1%	100.0%	97.5%
Prevalence	18.8%	37.5%	20.0%	5.0%	5.0%
Accuracy	78.8%	57.5%	82.5%	78.6%	97.5%

*Data are preliminary

** Amphetamine and methamphetamine were detected by a single test with Drugwipe

Discussion

Data collection during phase I was limited in part because the phlebotomy technician was inefficient and subsequently removed from his position. Although not specifically recorded, subjective discussions indicated that potential participants often refused because of less than enthusiastic recruitment.

Therefore, developing strategies to improve subject recruitment became a priority in future meetings of the local SLC partners and the ROSITA research partners (Tampa, Florida, Feb, 2004 and Santiago de Compostela, Spain, Nov, 2004).

Conclusions

Unfortunately, despite attempts during phase 1 to improve recruitment, only 41 subjects were enrolled. However; a complete set of on-site, laboratory-OF and laboratory-blood data were

collected. The Drugwipe performed acceptably having only a single failure reported. Cold winter-weather created storage and operational challenges because the devices were literally used “at the site” where temperatures could be below freezing.

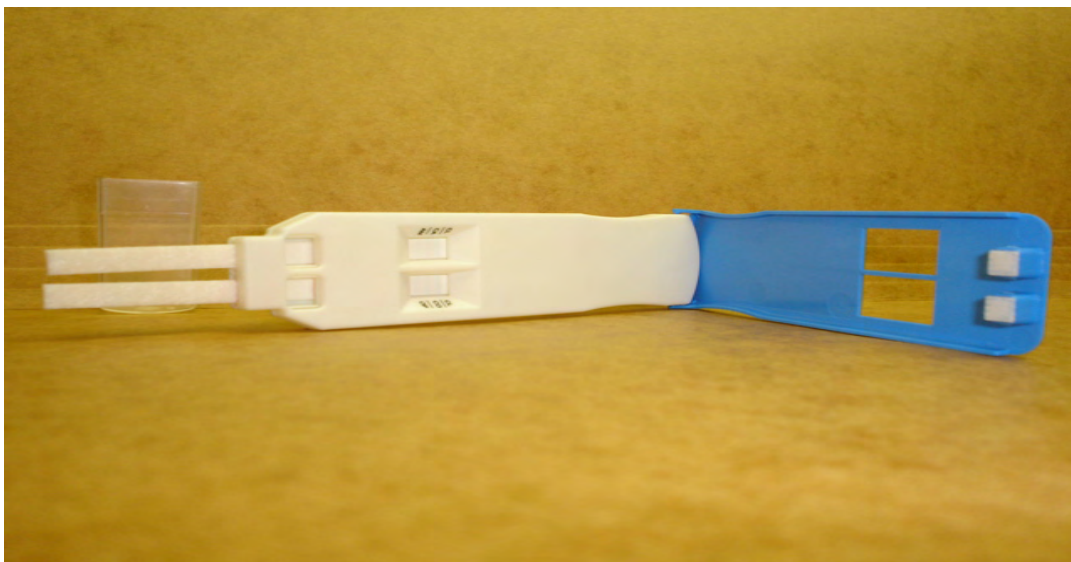
Phase II

Methods

Overview: Phase II of the SLC study began in March 2005. As in phase I, the goal was to collect 250 specimens during a nine-month study period (approximately 6-7 specimens each week). To improve enrollment numbers, three research technicians were hired and trained to recruit subjects. The technicians alternated nights and traveled with the SLCPD officers on Wednesday, Thursday, Friday and Saturday usually from 10 PM until 3 AM. The technicians were dressed in white laboratory coats and trained to approach, interview and obtain consent from participating subjects. They also collected the OF specimens, tested with the Drugwipe, completed the data sheets, shipped completed subject packs to The Walsh Group and delivered Intercept specimens to CHT for laboratory analysis. As of this report date, SLC has collected and tested 249 specimens on site with the Drugwipe. Six times that of specimens collected during phase I. Collections will continue through Feb, 2006. Laboratory testing has been completed on 210 samples.

Another significant change to the protocol was the elimination of the collection and testing of blood samples. These procedures were discontinued, therefore, results from phase II are from the Drugwipe on-site testing and the laboratory immunoassay screening and MS confirmations.

Device Assigned: For phase II, SLC was again assigned the Securetec Drugwipe.



Specimens collected: During phase II, a Drugwipe analysis is being performed on-site and an Intercept OF specimen is being collected for laboratory testing. No additional biological specimens are being collected.

Drugs to be tested: The SLC evaluation is comparing results from the laboratory OF (Intercept) tests to those obtained from the Drugwipe using the laboratory OF test results as the reference. Each specimen collected is analyzed for the following drugs:

Oral Fluids Rapid test: The Drugwipe tests OF for use of cannabis, cocaine, amphetamines, methamphetamines, and opiates.

Drugwipe test profile and cutoff concentrations

Amp	100 ng/mL	Cocaine	50 ng/mL
Meth	100 ng/mL	THC	30 ng/mL
Opiates	20 ng/mL	THC-COOH	3 ng/mL

Laboratory Oral Fluids Test (Intercept): CHT is screening the Intercept specimens for use of cannabis, cocaine, amphetamines, methamphetamines, opiates, and benzodiazepines. Confirmation testing using GC/MS or LC/MS/MS is conducted whenever there is a discrepancy between the Drugwipe and the OF laboratory results.

CHT Oral Fluids laboratory screening test profile (ng/mL buffer)

Amp/meth	50/25 ng/mL
Benzodiazepines	2 ng/mL
THC	2 ng/mL
Cocaine	8 ng/mL
Opiates	20 ng/mL

Inclusion Criteria: In compliance with the protocol, standard police measures were used to identify drivers suspected of being under the influence of alcohol or drugs. A driver was eligible for inclusion in the study if he/she was detained for any reason and the police officer had reasonable suspicion that the driver was operating the vehicle after using, or while under the influence of drugs. In phase II all drivers were(are) also eligible for inclusion if he/she was stopped for any reason and the subject volunteered to participate in the study.

Procedures: For drivers who meet the inclusion criteria for phase II, the research technician did the following: recruited them, obtained informed consent, collected the Intercept OF specimen and collected and tested with the Drugwipe.

Data Collection Form: All data required for the project are recorded on the Data Collection Form as described in phase I.

Consent Form: Procedures are same as used in phase I.

Subjects Summary Phase II (To Date)

	Phase II
Device Evaluated	(Securetec Drugwipe)
Number of Stops and subjects solicited	Not recorded*
Number of refusals	Not recorded*
Number of specimens collected/tested on-site	249
Number of device failures	2
Number of OF specimens collected with complete data	249/210 tested

* Data not recorded

Device Evaluation Summary Phase II (Preliminary*) (Device: Securetec Drugwipe)

	Total	THC	Coc	Meth/Amp	Opi
Total assays conducted "n"	990	244	248	249	249
True positives	37	13	13	6	5
True negatives	870	190	212	227	241
False positives	41	18	10	12	1
False negatives	42	23	13	4	2
Sensitivity	46.8%	41.0%	50.0%	60.0%	71.4%
Specificity	95.5%	91.3%	95.5%	95.0%	99.6%
Positive predictive value	47.4%	47.1%	56.5%	33.3%	83.3%
Negative predictive value	95.4%	89.2%	94.2%	98.3%	99.2%
Prevalence	8.0%	15.8%	10.5%	4.0%	2.8%
Accuracy	91.6%	83.4%	90.7%	93.6%	98.8%

*Laboratory analyses remain in progress

Discussion of Results

As a result of the measures taken to increase subject participation, sufficient subjects were recruited in phase II to meet the study goal of 250 subjects. However, subjects will continue to be recruited through February 2006. Of the 249 specimens collected, testing remains in process for nearly 40 samples. These data may have a significant impact on the final results of the study

Conclusions

The Drugwipe was considered acceptable for roadside testing based on its ease of use and willingness of the donors to provide specimens.

The Intercept was considered acceptable for roadside OF collections based on its ease of use and willingness of the donors to provide specimens.

In general, the Drugwipe was neither sensitive nor accurate enough to detect the two primary drugs of abuse commonly detected in DUID arrests, i.e. marijuana and cocaine (sensitivity 41 to 60%).

The TP, TN and FP rates for marijuana and cocaine were also unacceptable with the Drugwipe.

Acknowledgments. A special thanks to officers Roger Nielson, Tyler Reinwand, Robert Smith, Jeff Loosle, Steve Huffaker, Tom Wind, Rick Baldwin, Lonnie Martinez, Brandon Hansen, Jason Simpson, Rick Simpson, Jeff Kendrick and Tom Potter of the Salt Lake City Police Department for their participation in the project.

APPENDIX A

Intercept Oral Fluid Collection Procedures



Step 1

Inspect the expiration date on the Intercept® collection device packaging and open the outer packaging containing the collection pad and the specimen vial. Place the specimen vial on a flat surface in view of the donor. Instruct the donor to remove the handle and remove the collection device from the packaging sleeve.



Step 2

Instruct the donor to place the collection pad between the lower cheek and gums and gently rub the pad back and forth along the gum line until the pad is moist.



Step 3

Once moist, leave collection pad between cheek and gums for a full two (2) minutes.



Step 4*

After 2 minutes, have the donor open the specimen vial in an upright position, with the tip pointed downward, by gently rocking the cap back and forth to avoid spilling the contents. Instruct the donor to push the collection pad into the specimen vial as far as it will go.



Step 5*

Instruct the donor to snap the collection wand at the scored line against the side of the vial. Instruct the donor to not tilt the vial or spill the fluid inside.



Step 6*

Instruct the donor to place the cap onto the vial until it snaps, ensuring a secure fit.



Step 7*

Instruct donor to place tamper evident seal across the top of the specimen vial and down the sides, date and initial.

*At some sites Steps 4 through 7 were accomplished by the collector.

APPENDIX B

CASE NO: _____

OFFICER ID: _____

ROSITA2 DATA COLLECTION FORM

POLICE OFFICER INFORMATION

Date of Stop: _____

Time of Stop: _____

Site Location: HCSO

Reason For Drug Test:

- ☐ Unusual appearance, behavior, or odor indicating drug use
- ☐ Failed SFST and BAC level < 0.08
- ☐ Had drug / drug paraphernalia in vehicle
- ☐ BAC level inconsistent with impairment
- ☐ Driver admits to using drugs. Which drug(s)? _____

Reported BAC Test Results:

BAC Level	Time

Saliva Rapid Drug Test Device: Drugwipe

Indicate Positive Result with (+) and Negative with (-) for each drug

	AMP	METH	COC	THC	OPI
Test Results					

Specimen Collection Date/Time: Oral Fluid: _____
Collection Time

Urine: _____
Collection Time

Officers Comments: _____

FOR LABORATORY USE ONLY

Lab Accession Number: _____

Indicate Positive with (+) and Negative with (-) and quantitative concentration (ng/mL)

Drug	Confirmation Analyte	Oral Fluid		Urine	
		Screen	Confirm	Screen	Confirm
AMP	AMP / METH	(+) or (-)	ng/mL	(+) or (-)	ng/mL
	Amphetamine				
	Methamphetamine				
METH	MDMA (Ecstasy)				
	MDEA				
	MDA				
COC	COC				
	Cocaine				
	Benzoyllecgonine				
THC	THC				
	9THC (parent)				
	THCCOOH (metab.)				
OPI	OPI				
	Morphine				
	Codeine				
	Hydromorphone				
	Oxycodone				
BDP	Hydrocodone				
	BDP				
	Diazepam				
PCP	Oxazepam				
	PCP				
ETOH	(If Determined)				

Comments: _____

APPENDIX C

TITLE OF STUDY: ROSITA 2 – Evaluation of Oral Fluid (Saliva) Drug Detection Devices

You are being asked to be in a research study to test new drug detection devices that use saliva. To be in the study, all you need to do is sign this consent form and provide saliva specimens.

CONSENT TO BE IN THE RESEARCH STUDY

If I choose to consent and provide saliva specimens:

- ✓ All information that might tie my name to the results will be removed after all testing is completed. Therefore, after that is done my name cannot be connected to the results
- ✓ The specimens will be used for research purposes only
- ✓ The results will be kept completely confidential
- ✓ There are no known risks to my being in the study
- ✓ The saliva collection devices are sterile and the person collecting the saliva has been trained to do so in a manner that is safe
- ✓ The results of my saliva tests cannot be used against me in court
- ✓ My being in the study is completely voluntary
- ✓ Nothing will happen to me if I choose not to be in this study
- ✓ I may stop and quit the study at any time
- ✓ Because the scientific reliability of these saliva tests has not yet been established the results will be used for research purposes only

Knowing all this, I agree to be in this study and give you permission to collect saliva specimens from me for research purposes.

Research Subject – Sign Here: _____

Witness – Sign Here: _____

This project is being funded by the Office of National Drug Control Policy (ONDCP), The National Institute on Drug Abuse (NIDA), and The National Highway Traffic Safety Administration (NHTSA) under the direction of The Walsh Group, P.A. and in cooperation with this police agency.

If you have any questions about the research project, the risks involved, and/or your rights as a participant, you may contact us at the following number / address:

J. Michael Walsh, Ph.D., Principal Investigator
6701 Democracy Blvd., Suite 300
Bethesda, MD 20817
Telephone: 1-800-985-1225

Original – To Subject

Chapter 11: Evaluation of Roadside Oral Fluid Drug Testing Equipment/Devices

Site: Washington State, USA

Coordinator: Dr. J. Michael Walsh – U.S. Coordinator

Partners: Contributions of the Washington State Toxicology Laboratory, Washington State Patrol, County Sheriffs, and City Police across the state, Sgt. Steve Johnson (2003-2004), Dr. Dorota Schranz (2003-2004), Ann Marie Gordon, Lt. Robin Reichert.

Authors: Jayne E. Clarkson, Sgt. Carlos Rodriguez, Barry Logan, Ph.D., Leo Cangianelli, J. Michael Walsh, Ph.D.

Abstract

This study was a collaborative United States/European Union international effort to assess the prevalence of illegal drug use among drivers failing a standard field sobriety test [i.e. DUI suspects] and to evaluate the effectiveness of oral fluid drug detection technology to detect drug abuse by drivers at the roadside. The project was conducted in major cities in the U.S. and Western Europe by teams composed of scientists working in collaboration with local police. This paper presents analysis of data from one of the U.S. sites that participated in the larger study. The information was collected by the Washington State Toxicology Laboratory (WSTL) in Seattle Washington. Two oral fluid drug testing devices were evaluated by WSTL over a six month and later a seven month collection period with 174 specimens collected/tested. Unfortunately, the results of these analyses are incomplete at this time since the U.S. Phase II data collection is ongoing. The final results of Phase I and Phase II will be the subject of a final report published by all U.S. sites later in 2006.

Introduction

The Walsh Group in collaboration with Dr. Alain Verstraete [Ghent University, Belgium] established a collaborative agreement to conduct a joint U.S./European international study to assess the prevalence of illegal drug use among drivers failing a standard field sobriety test and determine the feasibility of police use of saliva drug testing devices at the roadside to verify drug use by drivers. This project was conducted in major cities in the U.S. and Western Europe by teams composed of scientists working in collaboration with local police. Intent of the project was to build on the success and the lessons learned in the original European ROSITA project that evaluated drug testing devices in eight European nations. For this project, four teams were identified in the United States located in Washington State, Salt Lake City, Utah, Wisconsin State and Hillsborough County, Florida. This section reports on the results of the study from the various law enforcement agencies across the State of Washington.

Under Washington Law a person can be found guilty of driving under the influence of any drug and is subject to punishment, if the person (1) was driving or was in actual physical control of a vehicle in Washington, and (2) while driving or in actual physical control of a vehicle, the defendant was under the influence of, or was affected by any drug OR (3) the defendant was

operating a vehicle in a negligent manner likely to endanger persons or property and while so operating the vehicle, exhibited the effects of having consumed a controlled substance or a prescription drug. Washington has an implied consent law for drugs which requires the suspect to provide a blood sample, and additionally a suspect may be compelled to provide a sample subject to a special evidence warning in the event that a person is killed or suffers a serious bodily injury as a result of a collision. Under these circumstances the person must be placed under arrest prior to the sample being taken. Washington law also permits a police officer to obtain a telephonic search warrant for blood based on probable cause of driving impairment and poor performance in sobriety tests. Washington's law currently has no provisions for the collection of an oral fluid specimen and for the purposes of this study, subjects were asked to provide a specimen voluntarily at the conclusion of the arrest and other testing procedures, with the understanding that the oral fluid test results would not be used in any criminal prosecution. Subjects signed a waiver to that effect.

Phase I

Methods

Overview:

During Phase I, training was offered to all certified Drug Recognition Expert (DRE) officers in Washington State. DRE officers receive specialized training in physiology, vital signs, and field sobriety testing in addition to the basic driving under the influence training that all officers in the state receive. This makes them better qualified to identify drug impaired drivers and categorize them into one or more of the seven classes of drugs, or to rule out possible medical or mental disorders that could have symptoms similar to drug intoxication. When a non-DRE officer contacts a subject who is suspected of driving under the influence of drugs, they will call out a DRE to evaluate the subject. This made DREs the ideal group of officers to train for this study, as they would encounter the most drug positive subjects. During Phase I, thirty-six DRE officers were trained. The training provided a brief history of the project, criteria for subject inclusion, and instructions on asking subjects if they would volunteer to participate, how to use the oral fluid collection devices assigned, and how to prepare the paper work for sample submission to the WSTL. Participating officers were made aware the goal for the project was 250 oral samples for Phase I.

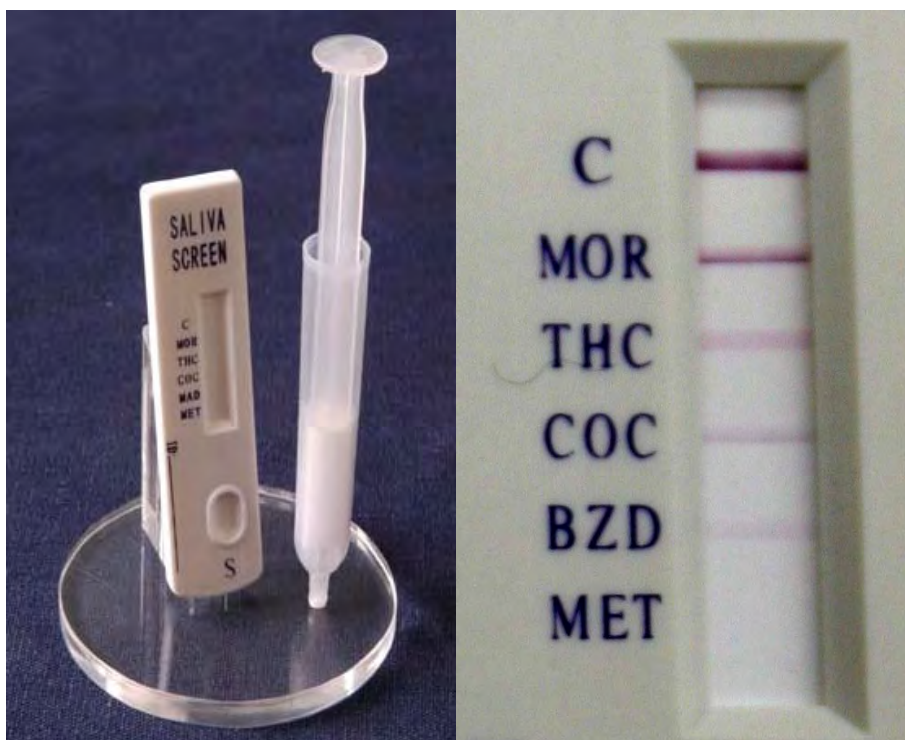
Specimens collected:

The WSTL collected two oral fluid specimens and blood. One oral fluid specimen was collected using the assigned experimental device. The second specimen was collected in the provided *Intercept*TM collection pad and forwarded in the transport vial to the University of Utah's Center for Human Toxicology (CHT) for laboratory confirmation. (See Appendix A for procedures involving collection of the *Intercept*TM specimen). A blood specimen was also collected as required by Washington State law for a DUID offense and was analyzed by the WSTL.

Device Assigned:

For Phase I, Washington State was assigned the *SalivaScreen 5*TM (UltiMed Products, Germany) drug test. This device is described in the following illustration.

UltiMed SalivaScreen 5TM



Matrix: Saliva, sweat

Available for: Methamphetamine, Opiates, Cocaine, Cannabinoids, and Benzodiazepines

Number of parameters per device: 5

Not FDA approved

Storage conditions: Room Temperature

Manipulations to obtain a result:

- 1) Collect the saliva sample with the collection swab.
- 2) Press the collection swab from top to bottom to expel 3-4 drops of collected saliva. Allow 2 minutes for dissolving of reagents and 10 minutes for the completion of the chromatography.
- 3) Test validity and results must be interpreted between 10 and 20 minutes after initiating the test.
- 4) Interpret the test validity.

Interpretation of the result: Visual [Although a reader device is available but not provided]

Save specimen for Confirmation: Possible in collection well

User friendliness: Good

Drugs to be tested:

The WSTL evaluation relied on the results of the laboratory saliva (*InterceptTM*) tests results and blood results to validate *SalivaScreen 5TM* drug testing system. The primary reference was the

laboratory saliva (*Intercept*TM) test results. Each specimen collected was tested for a core set of drugs as follows:

Saliva Rapid Test: The *SalivaScreen 5*TM device tested oral fluid for cannabis, cocaine, opiates, methamphetamines, and benzodiazepines.

SalivaScreen 5TM test profile and cutoffs

THC	20 ng/mL
Cocaine	30 ng/mL
Opiates	30 ng/mL
Methamphetamine	50 ng/mL
Benzodiazepines	50 ng/mL

Saliva Laboratory Test: CHT screened the laboratory saliva (*Intercept*TM) specimen for cannabis, cocaine, amphetamines, methamphetamines, opiates, and benzodiazepines.

CHT Oral Fluid laboratory screening test profile

THC	2 ng/mL
Cocaine	5 ng/mL
Amp/meth	40 ng/mL
Opiates	10 ng/mL
Benzodiazepines	2 ng/mL

Blood Laboratory Test: At minimum, the WSTL screened all blood samples for cannabis, cocaine, amphetamines, methamphetamines, opiates, and benzodiazepines using Enzyme Multiplied Immunoassay Technique (EMIT). Positive cases were confirmed by GC-MS.

WSTL blood laboratory screening test profile

THC	10 ng/mL
Carboxy THC	10 ng/mL
Benzoylcegonine	100 ng/mL
Methamphetamine	200 ng/mL*
Opiates	20 ng/mL
Benzodiazepines	100 ng/mL

* Samples that give an elevated reading, including those not above the cutoff, are confirmed by GC-MS.

Inclusion Criteria:

Before a driver was invited to participate in the study, they had to meet at least one of the following criteria:

- The driver's appearance, behavior or odor indicated drugs may have been used
- The driver's Breath Alcohol Concentration was less than 0.08 or inconsistent with the level of observed impairment
- The driver had illicit drugs or drug paraphernalia in the vehicle
- The driver admitted to using drugs

Procedures:

After subjects were placed under arrest and the officer took the subject to the hospital for a legal blood draw, subjects were invited to participate in the study. All volunteers signed a consent form before collection of the oral fluid samples.

1. One oral fluid specimen was tested by the DRE officer using the *SalivaScreen 5TM* on-site drug testing device and a second oral fluid specimen was collected using the *InterceptTM* saliva collector. The results of the *SalivaScreen 5TM* device were read by the police officer and recorded on the data sheet. The second *InterceptTM* specimen was annotated with the case number on the *InterceptTM* saliva transport vial and placed in a plastic envelope along with the data and consent sheets. This completed subject package was submitted with the blood tubes to the WSTL.
2. Upon arrival at the WSTL, blood specimens were handled in accordance with normal Washington State procedures. The paperwork specific to the study and the *InterceptTM* devices were transferred to the laboratory's ROSITA coordinator, who then submitted the *InterceptTM* devices to CHT and then documented results of the testing.

Data Collection Form:

All data required for the project was written on the Data Collection Form as described below. A copy is attached as Appendix (B).

Case No. – A unique case number generally same as used for the arrest citation

Officer ID – Officer's badge number or other officer identification

Date of Stop: (Month/day/year)

Time of Stop: Time of the stop (i.e. 9:45 pm)

Site Location – Preprinted on the form for each center

Reason for Drug Test – Checked box indicating the reason for the drug test

Reported BAC Test Results – Reported BAC level and time the BAC test was conducted

Oral Fluid Drug Test Device – The name of the assigned saliva rapid drug-testing device

Officer's Test Results – Results indicating "+" or "-" for each drug tested

Specimen Collection Time – Time each of the specimen(s) were collected

Consent Form:

A copy of the standard U.S. site consent form is included as Appendix (C). Each subject included in the study was asked to sign a consent form prior to participating and prior to collecting the specimen(s). Normally, this was completed after all evidentiary specimens and forms for the arrest were concluded by the police officer.

Results Summary

Subjects Summary Phase I	Phase I
Device Evaluated	UltiMed Products, <i>SalivaScreen 5TM</i>
Number of stops and subjects solicited	Unknown
Number of refusals	Unknown
Number of specimens collected/tested	70
Number of device failures	33
Number of OF specimens collected with complete data	37
Number of blood specimens collected	68

Device Evaluation Summary, Phase I (Device: UltiMed Products, *SalivaScreen 5TM*)

	Total	THC	Amph	Coc	Benzo	Opi
Total assays conducted "n"	185	37	37	37	37	37
True Positives	28	6	7	9	2	4
True Negatives	132	17	28	25	32	30
False Positives	5	2	0	0	2	1
False Negatives	20	12	2	3	1	2
Sensitivity	58.33%	33.33%	77.78%	75.00%	66.67%	66.67%
Specificity	96.35%	89.47%	100.00%	100.00%	94.12%	96.77%
Positive predictive value	84.85%	75.00%	100.00%	100.00%	50.00%	80.00%
Negative predictive value	86.84%	58.62%	93.33%	89.29%	96.97%	93.75%
Prevalence	25.95%	48.65%	24.32%	32.43%	8.11%	16.22%
Accuracy	86.49%	62.16%	94.59%	91.89%	91.89%	91.89%

Discussion

There were several difficulties encountered in collecting valid data during Phase I. One of the problems encountered was poor officer participation. Only twenty of the thirty-six officers trained submitted samples. Of those twenty who submitted samples, only half submitted more than one sample. The lack of participation may have been the result of an inadequate sample collection device. The test kits were required to be kept at room temperature. The eastern portion of Washington State has large seasonal variations in temperature and during the winter months the temperature is below the recommended storage temperature of 2°C. This prevents those officers from carrying the devices in their car. Additionally, the *SalivaScreen 5TM* device gave more invalid tests than it did valid. Twenty-one devices did not give readings (control bar did not appear). Other officers reported the result bands smearing or not enough saliva collected by the device to give a reading. The lack of valid data may have discouraged officers from asking other subjects to volunteer. Feedback from officers also indicated that the device was not practical and needed to be simplified. Specifically, that it took two minutes to collect the

sample, having to manually transfer saliva from collection device to reading device, and then waiting an additional ten minutes for the device to produce results.

Conclusions

At the end of Phase I only thirty-seven valid specimens were collected. Due to the problems encountered with the *SalivaScreen 5TM* device, Washington State switched devices after Phase I.

Phase II

Methods

Overview:

During Phase II, Washington State changed the selection criteria for who would receive training. This time, the ROSITA training was offered to both DRE officers and non-DRE officers that specialize in the area of DUI enforcement or narcotics enforcement, and were willing to participate in the ROSITA project. Thirty-three officers received training.

In an effort to increase the number of samples received, during the sixth month of Phase II it was decided to ask subjects that were participants in DRE field certifications to volunteer specimens. During field certifications students training to become DREs examine subjects that may be under the influence of a drug. These subjects are usually parole violators that have volunteered to be examined. After the students completed their examination, the volunteers were asked to participate in the ROSITA project.

Specimens collected:

Specimens collected in Phase II were similar to Phase I, with the exception of the assigned device. Again, the WSTL collected two oral fluid specimens and blood. One oral fluid specimen was collected using the assigned experimental device. The second specimen was collected in the provided *InterceptTM* collection pad and forwarded in the transport vial to the University of Utah's Center for Human Toxicology (CHT) for laboratory confirmation. A blood specimen was also collected as required by Washington State law for a DUID offense and was analyzed by the WSTL.

Device Assigned:

For Phase II, Washington State was assigned the Securetec *DrugWipe5TM* drug test. This device is described in the following illustration.

Securetec DrugWipe5TM



Matrix: Saliva, sweat, wipe

Available for: Amphetamines, Opiates, Cocaine, and Cannabinoids

Number of parameters per device: 4

Not FDA approved

Storage conditions: Room temperature

Manipulations to obtain a result:

- 1) Disconnect wiping section from the device
- 2) Wipe the surface of the tongue for approximately 10 seconds
- 3) Reassemble the device and dip the absorbent pad into water for 15 seconds
- 4) Read the result after approximately 10 minutes

Interpretation of the result: Visual

Save specimen for Confirmation: Possible

User friendliness: Good

Drugs to be tested:

In Phase II the WSTL evaluation again relied on the results of the laboratory saliva (*InterceptTM*) tests results and blood results to validate the *DrugWipe5TM* forensic drug testing system. The primary reference was again the laboratory saliva (*InterceptTM*) test results. Each specimen collected was tested for a core set of drugs as follows:

Saliva rapid Test: The *DrugWipe5TM* device tested oral fluid for cannabis, cocaine, opiates, and amphetamines.

DrugWipe5TM test profile and cutoffs

THC	30 ng/mL
Cocaine	50 ng/mL
Opiates	20 ng/mL
Methamphetamine	100 ng/mL

Laboratory saliva Test (InterceptTM): CHT screened the laboratory saliva (InterceptTM) specimen for cannabis, cocaine, amphetamines, methamphetamines, and opiates.

CHT Oral Fluid laboratory screening test profile

THC	2 ng/mL
Cocaine	5 ng/mL
Amp/meth	40 ng/mL
Opiates	10 ng/mL
Benzodiazepines	2 ng/mL

Blood laboratory Test: At minimum, the WSTL screened all blood samples for cannabis, cocaine, amphetamines, methamphetamines, and opiates using Enzyme Multiplied Immunoassay Technique (EMIT). Positive cases were confirmed by GC-MS.

WSTL blood laboratory screening test profile

THC	10 ng/mL
Carboxy THC	10 ng/mL
Benzoylcegonine	100 ng/mL
Methamphetamine	200 ng/mL*
Opiates	20 ng/mL

* Samples that give an elevated reading, including those not above the cutoff, are confirmed by GC-MS.

Inclusion Criteria:

All drivers contacted and placed under arrest for DUID were eligible to participate in the study. Additionally, drivers who were under arrest for DUI and provided high breath alcohol samples but showed signs of drug impairment were also invited to participate. Finally, any parole violator that participated in the DRE Field Certifications was eligible. All of the subjects that participated were volunteers and signed a consent form.

Procedures:

For drivers, who met the inclusion criteria, specimens were collected and handled the same as they were for Phase I. For volunteers recruited from the DRE Field Certifications, a phlebotomist was on site to collect voluntary blood samples in addition to the oral fluids

collected by the officers. These samples were forwarded to the WSTL in a similar manner to the legal blood draws.

Data Collection Form:

All data required for the project was written on the Data Collection Form as described in Phase I.

Consent Form:

Forms and procedures used for Phase II were the same as those used for Phase I.

Results Summary (for data received in 2005)

Subjects Summary Phase II	Phase II
Device Evaluated	Securetec, <i>DrugWipe5TM</i>
Number of stops and subjects solicited	Unknown
Number of refusals	Unknown
Number of specimens collected/tested	104
Number of device failures	0*
Number of OF specimens collected with complete data	64 **
Number of blood specimens collected	100

*This is for the *DrugWipe5TM* only. One *InterceptTM* device failed to collect enough sample to allow for testing.

**Includes 3 cases where blood was refused but all other testing was complete, but does not include specimens received where testing has not yet been completed.

Device Evaluation Summary, Phase II (Device: Securetec *DrugWipe5TM*)

	Total	Cannabinoids	Amphetamines	Cocaine	Opiates
Total assays conducted "n"	256	64	64	64	64
True Positives	37	4	10	17	6
True Negatives	166	37	46	33	50
False Positives	4	1	1	2	0
False Negatives	49	22	7	12	8
Sensitivity	43.02%	15.38%	58.82%	58.62%	42.86%
Specificity	97.65%	97.37%	97.87%	94.29%	100.00%
Positive predictive value	90.24%	80.00%	90.91%	89.47%	100.00%
Negative predictive value	77.21%	62.71%	86.79%	73.33%	86.21%
Prevalence	46.49%	40.63%	26.56%	45.31%	21.88%
Accuracy	109.73%	64.06%	87.50%	78.13%	87.50%

Discussion

The device failure rate was much lower for Phase II. To date, none of the *DrugWipe5TM* devices has failed to give a reading. However, one of the *InterceptTM* reference collection devices did not collect enough fluid to provide valid results.

We have experienced greater officer participation during Phase II. This is due in part to the inclusion of DRE Certification Subjects being allowed to volunteer for the study. Unfortunately, during one of the certification trainings, a set of the *InterceptTM* devices were discarded before submission to the WSTL. Another problem encountered during the collections at the training was running out of the *DrugWipe5TM* kits.

Overall, feedback from officers that use the device is positive. They report the device is easy to handle, the oral fluids are gathered quickly and it is not very intrusive. There have not yet been complaints of the water packets freezing (*DrugWipe5TM* test kits are required to be kept at room temperature) but with the cold weather recently experienced, this is a potential cause for concern.

The primary complaint received is the high cut-off rate for THC. In Washington State, marijuana is the most frequently detected drug, other than ethanol, detected in arrests made for impaired driving. The officers feel that a sensitivity of 15% is not acceptable.

Conclusions

The *SalivaScreen 5TM* used in Phase I was not suitable for roadside testing for two primary reasons:

- The device suffered a large number of failures (they failed to run to completion) that discouraged the police from using them.
- The device was not sensitive and accurate enough to detect the marijuana, which is the most frequently encountered drug (other than ethanol) in Washington State.

As a result, Washington switched devices during Phase II, and began using the *DrugWipe5TM*. Phase II in Washington State will continue through June 2006.

The *DrugWipe5TM* used in Phase II is superior to the *SalivaScreenTM* for several reasons:

- The *DrugWipe5TM* did not have lines that smeared, making interpretation of the results *DrugWipe5TM* results easier and more reliable.
- The *DrugWipe5TM* was easier for officers to use. Results were able to be read within ten minutes, and they did not have to manually extract saliva out of the device.
- The *DrugWipe5TM* reliably gave control lines, indicating that the device had worked.

However, the ability to detect marijuana is still not sensitive enough for Washington State. The ability of the *DrugWipe5TM* to detect cocaine is also questionable.

Overall, in both Phase I and Phase II, the laboratory saliva collected using the *InterceptTM* device worked better than the on-site rapid tests.

APPENDIX A

Intercept Oral Fluid Collection Procedures



Step 1

Inspect the expiration date on the Intercept® collection device packaging and open the outer packaging containing the collection pad and the specimen vial. Place the specimen vial on a flat surface in view of the donor. Instruct the donor to remove the handle and remove the collection device from the packaging sleeve.



Step 2

Instruct the donor to place the collection pad between the lower cheek and gums and gently rub the pad back and forth along the gum line until the pad is moist.



Step 3

Once moist, leave collection pad between cheek and gums for a full two (2) minutes.



Step 4*

After 2 minutes, have the donor open the specimen vial in an upright position, with the tip pointed downward, by gently rocking the cap back and forth to avoid spilling the contents. Instruct the donor to push the collection pad into the specimen vial as far as it will go.



Step 5*

Instruct the donor to snap the collection wand at the scored line against the side of the vial. Instruct the donor to not tilt the vial or spill the fluid inside.



Step 6*

Instruct the donor to place the cap onto the vial until it snaps, ensuring a secure fit.



Step 7*

Instruct donor to place tamper evident seal across the top of the specimen vial and down the sides, date and initial.

*At some sites Steps 4 through 7 were accomplished by the collector.

APPENDIX B

CASE NO: _____

OFFICER ID: _____

ROSITA2 DATA COLLECTION FORM

POLICE OFFICER INFORMATION

Date of Stop: _____

Time of Stop: _____

Site Location: Washington

Reason For Drug Test:

- ☐ Unusual appearance, behavior, or odor indicating drug use
- ☐ Failed SFST and BAC level < 0.08
- ☐ Had drug / drug paraphernalia in vehicle
- ☐ BAC level inconsistent with impairment
- ☐ Driver admits to using drugs. Which drug(s)? _____

Reported BAC Test Results:

BAC Level	Time

Saliva Rapid Drug Test Device: DrugWipe 5

Indicate Positive Result with (+) and Negative with (-) for each drug

	CA	AM	CO	OP
Test Results				

Specimen Collection Date/Time: Oral Fluid: _____ Urine: _____
Collection Time Collection Time

Officers Comments: _____

For Laboratory Use Only

Lab Accession Number: _____

Indicate Positive with (+) and Negative with (-) and quantitative concentration (ng/mL)

Drug	Confirmation Analyte	Oral Fluid		Urine	
		Screen	Confirm	Screen	Confirm
AMP	AMP / METH	(+) or (-)	ng/mL	(+) or (-)	ng/mL
	Amphetamine				
	Methamphetamine				
METH	MDMA (Ecstasy)				
	MDEA				
	MDA				
COC	COC				
	Cocaine				
	Benzoyllecgonine				
THC	THC				
	9THC (parent)				
	THCCOOH (metab.)				
OPI	OPI				
	Morphine				
	Codeine				
	Hydromorphone				
	Oxycodone				
BDP	BDP				
	Diazepam				
	Oxazepam				
PCP	PCP				
ETOH	(If Determined)				

Comments: _____

APPENDIX C

TITLE OF STUDY: ROSITA 2 – Evaluation of Oral Fluid (Saliva) Drug Detection Devices

You are being asked to be in a research study to test new drug detection devices that use saliva. To be in the study, all you need to do is sign this consent form and provide saliva specimens.

CONSENT TO BE IN THE RESEARCH STUDY

If I choose to consent and provide saliva specimens:

- ✓ All information that might tie my name to the results will be removed after all testing is completed. Therefore, after that is done my name cannot be connected to the results
- ✓ The specimens will be used for research purposes only
- ✓ The results will be kept completely confidential
- ✓ There are no known risks to my being in the study
- ✓ The saliva collection devices are sterile and the person collecting the saliva has been trained to do so in a manner that is safe
- ✓ The results of my saliva tests cannot be used against me in court
- ✓ My being in the study is completely voluntary
- ✓ Nothing will happen to me if I choose not to be in this study
- ✓ I may stop and quit the study at any time

Knowing all this, I agree to be in this study and give you permission to collect saliva specimens from me for research purposes.

Research Subject Sign Here: _____

Witness – Sign Here: _____

This project is being funded by the Office of National Drug Control Policy (ONDCP), The National Institute on Drug Abuse (NIDA), and The National Highway Traffic Safety Administration (NHTSA) under the direction of The Walsh Group, P.A. and in cooperation with this police agency.

If you have any questions about the research project, the risks involved, and/or your rights as a participant, you may contact us at the following number / address:

J. Michael Walsh, Ph.D., Principal Investigator
6701 Democracy Blvd., Suite 300
Bethesda, MD 20817
Telephone: 1-800-985-1225

Original – To Subject

Chapter 12: Evaluation of Roadside Oral Fluid Drug Testing Equipment/Devices - ROSITA 2: Wisconsin Site Report for Phase I

Authors: Laura J. Liddicoat, B.S. and Patrick Harding, B.S., Toxicology Section, Wisconsin State Laboratory of Hygiene

Abstract

A select group of officers from eleven Wisconsin law enforcement agencies were trained as researchers to recruit and test drivers arrested for driving under the influence of drug (DUID) offenses. After all standard arrest procedures and collection of evidentiary blood samples (if any) had been completed the subjects were invited to participate in a study to evaluate oral fluid drug testing devices. The officers tested consenting subjects with either the RapiScan (Cozart) or OraLab (Varian, Inc.) device. Subjects then provided a confirmatory oral fluid sample using the Intercept (OraSure Technologies, Inc.) collection device and an optional confirmatory urine sample. The confirmatory oral fluid samples were forwarded to The Center for Human Toxicology (CHT), Salt Lake City, Utah, for testing. Blood and urine drug testing were conducted by the Wisconsin State Laboratory of Hygiene Toxicology Section. During Phase I of the study a total of 57 subjects (out of a target number of 250) participated; 40 subjects were tested with the RapiScan device and 17 were tested using the OraLab device. The on-site oral fluid results were compared to laboratory results from the paired confirmatory oral fluid, blood and urine specimens, as available.

This study was part of a collaborative US/EU international effort to assess the prevalence of illegal drug use among drivers failing standard field sobriety tests and to evaluate the effectiveness of oral fluid drug detection technology to detect drug abuse by drivers at the roadside.

Introduction

Wisconsin was selected as one of four US sites for participation in the ROSITA 2 oral fluid drug detection research project. The Wisconsin State Laboratory of Hygiene (WSLH) Toxicology Section served as the coordinating agency for this site. Funding for conducting the research was coordinated by The Walsh Group. A research protocol incorporating the ROSITA 2 established research guidelines was submitted to the University of Wisconsin Institutional Review Board (IRB) for approval. After numerous revisions the protocol was approved on Feb 25, 2004, with Phase I data collection beginning March 11, 2004 and continuing through the end of December 2004. A select group of law enforcement officers from eleven agencies in five Wisconsin counties received special researcher training to recruit and test subjects for this study (see Appendix A). Subjects meeting the selection criteria were asked by the officer-researchers to participate in the study and were given an information sheet explaining the purpose of the research, the testing protocol and assurances that they would not be exposed to risk or that the results of the research could not be used against them in any way. The information sheet used in Wisconsin is shown in Appendix B. The District Attorneys Office of each county participating in this study provided a statement ensuring that oral fluid drug test results would not be used in any prosecution of the test subjects.

Wisconsin impaired driving statutes in effect during Phase I of this study prohibit the use of an intoxicant, controlled substance, drug, or any other substance that affects the driver's ability to safely operate a motor vehicle. In December 2003 a statute was enacted making it illegal to operate a motor vehicle with any detectable amount of a "restricted controlled substance" in his or her blood. Restricted controlled substances are defined as all Schedule 1 substances (with cannabinoids defined solely as Delta-9-tetrahydrocannabinol), methamphetamine, cocaine and cocaine metabolites. Blood is the only specimen defined for this statute. The WSLH provides evidence kits to law enforcement and medical facilities to enable blood specimen collection under applicable Wisconsin statutes.

Methods

Oral Fluid Drug Testing Devices:

Two different devices were assigned to the Wisconsin sites. The RapiScan (Cozart Bioscience LTD) device was assigned to the officers in two counties and the OraLab (Varian, Inc.) to the remaining three counties. Devices are described below with the parameters utilized in the Wisconsin ROSITA 2 protocol.

RapiScan: (See Appendix C)

Matrix: Saliva

Target Drugs & Listed Cut-offs (ng/mL):

Benzodiazepines (30), d-Amphetamine (45),
Δ9-THC (150), Cocaine (30) & Morphine (30)

Storage conditions: Room temperature

Operating Instructions:

1. The collection pad is placed in the donor's mouth for specimen collection until a blue indicator appears.
2. Transfer the collection pad into the transport tube. The transport tube contains a buffered solution that facilitates dissolution of the sample pad contents.
3. Cap the transport tube and tap tube to separate the collection pad from the stem. Specimen is stable at this point for 72 hours and may be analyzed at any point during this time period.
4. Remove cap and stem from transport tube.
5. Insert the separator filter (similar to a serum separator) fully into the transport tube.
6. Transfer six drops of the filtered and buffered saliva into the cassette sample-well.
7. Wait approximately 2-30 seconds until the reagent appears on the test membrane.
8. Insert the cassette into the instrument and automatically read results after 12 minutes.

Interpretation of the results: Digital read-out



OraLab: (See Appendix D)

Matrix: Saliva

Target Drugs & Listed Cut-offs (ng/mL):

Cocaine (20), Morphine (40), d-Amphetamine (50),
d-Methamphetamine (50), Phencyclidine (10) & Δ9-THC (100)

Storage conditions: Room temperature

Operating Instructions:

1. The oral fluid sample is collected from the donor using a foam collector.
2. To initiate a test, the collector is placed into the sample well of the device and pushed down slowly to transfer the oral fluid from the collector into the test cassette. Oral fluid that is not absorbed by the test cassette-strips overflows into a reservoir that can be sealed and sent to a laboratory for alternate testing [e.g. confirmation testing].



3. Test validity and drug-test results were read visually between 10 and 15 minutes after initiating the test.

Interpretation of result: Visual

Researcher training:

Police officers were selected based on their training as drug recognition experts and/or their activity in arresting impaired drivers. WSLH personnel provided on site training to officers in conducting ethical research, properly recruiting subjects, use of the oral fluid drug testing devices, collection of confirmatory oral fluid samples, collection of confirmatory urine samples, completion of research-related paperwork and specimen packaging. Additionally, the University of Wisconsin IRB required officers to complete a web-based human subject's course and be officially approved as unaffiliated researchers prior to recruiting and testing subjects. A total of 45 officers from 11 police agencies completed all training requirements.

Subject Inclusion Criteria:

Standard police measures were used to identify drivers suspected to be under the influence of alcohol and/or drugs. Subjects who were arrested by a law enforcement officer for operating a motor vehicle while impaired were asked to participate in ROSITA 2 after all standard arrest procedures had been completed. This was done to ensure that the results of the field oral fluid drug testing could not influence or jeopardize the arrest or prosecution of the case. To qualify for participation in this study a subject had to be 18 years of age or older, stopped by a law enforcement officer for a driving or vehicle equipment infraction and the officer must have had reasonable suspicion of drug use. For the purposes of this study the criteria for reasonable suspicion were any of the following:

1. Breath alcohol concentration (BrAC) < 0.08 g/210L
2. Driver admission of drug use
3. Drugs or paraphernalia found on the subject or in the vehicle they were driving
4. BrAC inconsistent with observed impairment

Subject Testing Procedures:

Subjects who met the above criteria were asked to participate only after the officer had completed all arrest procedures, including field sobriety tests, examination by a drug recognition expert (when conducted), evidentiary breath alcohol test (if any) and collection of a blood sample for analysis of alcohol and other drugs (if any). An information sheet (Appendix B) describing the research protocol and subject safeguards was provided to consenting subjects. Subjects were free to withdraw from participation at any time.

Oral fluid specimens were obtained and tested on-site by officer-researchers according to the manufacturers' recommended procedures. Test results were interpreted by the officer and recorded on the data form (Appendix E – RapiScan; Appendix F - OraLab). A second confirmatory oral fluid specimen was collected using the Intercept (OraSure Technologies, Inc.) collection device and a confirmatory urine specimen (unwitnessed) was also obtained when possible. Officers completed the data form, labeled confirmatory specimens with ROSITA 2 case labels, packaged them in the Styrofoam kit and mailed it back to WSLH.

ROSITA 2 Specimen Collection Kits:

The WSLH prepared and distributed sample collection kits to participating officers at the initial training and as needed during Phase I. The kits contained an Intercept oral fluid confirmatory sample collection device, a screw top plastic container for a confirmatory urine sample, ROSITA 2 case labels, data form, subject information sheet, packaging materials and

shipping labels. The kits were provided in Styrofoam containers prepared with mailing labels and prepaid postage for shipment to the WSLH.

Confirmatory Specimens:

Oral Fluid Samples:

Confirmatory oral fluid samples were collected using the Intercept device (Appendix G). The collection pad from this device was placed in the subject's mouth for 3-5 minutes, along the sides and lower margins of the tongue. The collection pad was then placed in the specimen vial, the handle broken off and the vial capped. A label with the ROSITA 2 case number was then affixed to the vial.

Blood Samples:

Blood specimens were available only when obtained as part of the arrest procedure, using materials provided in WSLH Implied Consent Blood kits. Blood was collected into two 10-mL vacuum tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate. A ROSITA 2 case number label was affixed to the outside of the shipping container serving as the only ROSITA 2 identification associated with the specimens inside. All identifiers connecting the evidentiary blood specimen to the ROSITA 2 research specimens were removed upon completion of testing.

Urine Samples:

A plastic container was provided for the subject to provide an optional, unwitnessed urine specimen. The sample container was labeled with the ROSITA 2 case number and packaged for shipping to WSLH with the oral fluid specimen.

Laboratory Drug Testing Procedures and Methods:

Oral Fluid: Specimens collected with the Intercept device were frozen upon receipt at WSLH and shipped periodically to The Center for Human Toxicology (CHT), Salt Lake City, Utah, for testing. Initial screening was by enzyme immunoassay, with repeat testing by the same technique or GC/MS when discrepant results between the rapid on-site device and the confirmatory test occurred. Table 1 lists the drugs included in the testing protocol and their cut-off concentrations.

Blood: All blood specimens for this research were evidentiary samples submitted to and tested at WSLH in conjunction with an impaired driving arrest. The WSLH comprehensive blood drug screening protocol included analysis by enzyme immunoassay and examination of an alkaline extract by gas chromatography with nitrogen phosphorous detector (GC/NPD). All presumptive positive results were confirmed by mass spectrometry (GC/MS). The WSLH drug testing procedures are able to detect all drugs specified in Table 1 at the listed cutoff concentrations.

Urine: Urine specimens were screened by enzyme immunoassay. The drug classes tested with their respective cut-off concentrations are listed below. The WSLH standard practice of confirming presumptive positive results by mass spectrometry analysis was omitted per the US ROSITA 2 protocol.

Amphetamines	500 ng/mL	Cocaine (Metabolite)	300 ng/mL
Barbiturates	200 ng/mL	Opiates	300 ng/mL
Benzodiazepines	200 ng/mL	Phencyclidine	25 ng/mL
Cannabinoids	20 ng/mL		

Table 1: Laboratory Drug Testing Parameters

Substance	Blood - Cut-Off Concentration (ng/mL)	Oral Fluid - Cut-Off Concentration (ng/mL)
Amphetamine	20	25
Methamphetamine	20	25
MDMA	20	25
MDA	20	25
MDEA	20	25
Cocaine	20	4
Benzoyllecgonine	20	4
6-acetylmorphine	2	2
Morphine	10	20
Codeine	10	20
Other opiates	10	20
Methadone	20	20
Δ^9 -Tetrahydrocannabinol	1	2
THCCOOH	5	Not Tested
11-OH-THC	1	1
Diazepam	50	0.5
Nordiazepam	50	0.5
Oxazepam	50	0.5
Temazepam	50	0.5
7-Aminoflunitrazepam	10	0.2
Alprazolam	50	0.5
Lorazepam	50	0.5
Clonazepam	50	0.2

Results Summary

Subject Summary:

Fifty-seven subjects were recruited and tested in Phase I. The summary information and individual device numbers are listed in Table 2. Although the total number of subjects was low, a high percentage of subjects tested positive for one or more drugs. Of the 57 subjects tested, 51 (89.5%) had confirmed positive results for one or more drugs.

Table 2: Subject Summary Data

Subjects Summary Phase I	OralLab	RapiScan
Number of stops and subjects solicited	Unknown	Unknown
Number of refusals	Unknown	Unknown
Number of specimens collected/tested	17	40
Number of device failures	5	0
Confirmatory oral fluid specimens collected w/complete data	14	40
Confirmatory blood specimens collected w/complete data	8	35
Confirmatory urine specimens collected w/complete data	13	35

Test Device Data:

The following data tables provide a summary of the oral fluid field test results as compared to the results of the confirmatory specimens.

Table 3: Results of on-site OraLab device as compared to results in oral fluid

Summary: OraLab vs Oral Fluid (CHT)

Analyte	TOTAL	CANNABIS	AMPHETAMINES	METHAMP	COCAINE	OPIATES
Total n	66	12	14	12	14	14
TP	8	3	0	1	3	1
TN	49	4	13	11	8	13
FP	3	0	1	0	2	0
FN	6	5	0	0	1	0
Sensitivity	57.1 %	37.5 %	NA	100.0 %	75.0 %	100.0 %
Specificity	94.2 %	100.0 %	92.9 %	100.0 %	80.0 %	100.0 %
Positive predictive value	72.7 %	100.0 %	0.0 %	100.0 %	60.0 %	100.0 %
Negative predictive value	89.1 %	44.4 %	100.0 %	100.0 %	88.9 %	100.0 %
Prevalence	21.2 %	66.7 %	0.0 %	8.3 %	28.6 %	7.1 %
Accuracy	86.4 %	58.3 %	92.9 %	100.0 %	78.6 %	100.0 %

Table 4: Results of on-site OraLab device as compared to results in blood

Summary: OraLab vs Blood

Analyte	TOTAL	CANNABIS	AMPHET	METHAMP	COCAINE	OPIATES	PCP
Total n	43	7	8	6	8	8	6
TP	4	2	0	0	1	1	0
TN	34	3	7	5	6	7	6
FP	3	0	1	1	1	0	0
FN	2	2	0	0	0	0	0
Sensitivity	66.7 %	50.0 %	NA	NA	100.0 %	100.0 %	NA
Specificity	91.9 %	100.0 %	87.5 %	83.3 %	85.7 %	100.0 %	100.0 %
Positive predictive value	57.1 %	100.0 %	0.0 %	0.0 %	50.0 %	100.0 %	NA
Negative predictive value	94.4 %	60.0 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %
Prevalence	14.0 %	57.1 %	0.0 %	0.0 %	12.5 %	12.5 %	0.0 %
Accuracy	88.4 %	71.4 %	87.5 %	83.3 %	87.5 %	100.0 %	100.0 %

Table 5: Results of on-site OraLab device as compared to results in urine

Summary: OraLab vs Urine

Analyte	TOTAL	CANNABIS	AMPHET	METHAMP	COCAINE	OPIATES	PCP
Total n	72	11	13	11	13	13	11
TP	7	2	0	1	3	1	0
TN	51	3	10	9	7	11	11
FP	3	0	1	0	2	0	0
FN	11	6	2	1	1	1	0
Sensitivity	38.9 %	25.0 %	0.0 %	50.0 %	75.0 %	50.0 %	NA
Specificity	94.4 %	100.0 %	90.9 %	100.0 %	77.8 %	100.0 %	100.0 %
Positive predictive value	70.0 %	100.0 %	0.0 %	100.0 %	60.0 %	100.0 %	NA
Negative predictive value	82.3 %	33.3 %	83.3 %	90.0 %	87.5 %	91.7 %	100.0 %
Prevalence	25.0 %	72.7 %	15.4 %	18.2 %	30.8 %	15.4 %	0.0 %
Accuracy	80.6 %	45.5 %	76.9 %	90.9 %	76.9 %	92.3 %	100.0 %

Table 6: Results of on-site RapiScan device as compared to results in oral fluid

Summary: RapiScan vs Oral Fluid (CHT)

Analyte	TOTAL	CANNABIS	AMPHETAMINES	OPIATES	COCAINE	BENZOS
Total n	200	40	40	40	40	40
TP	27	13	2	4	6	2
TN	134	14	28	34	29	29
FP	20	6	7	0	2	5
FN	19	7	3	2	3	4
Sensitivity	58.7 %	65.0 %	40.0 %	66.7 %	66.7 %	33.3 %
Specificity	87.0 %	70.0 %	80.0 %	100.0 %	93.5 %	85.3 %
Positive predictive value	57.4 %	68.4 %	22.2 %	100.0 %	75.0 %	28.6 %
Negative predictive value	87.6 %	66.7 %	90.3 %	94.4 %	90.6 %	87.9 %
Prevalence	23.0 %	50.0 %	12.5 %	15.0 %	22.5 %	15.0 %
Accuracy	80.5 %	67.5 %	75.0 %	95.0 %	87.5 %	77.5 %

Table 7: Results of on-site RapiScan device as compared to results in blood

Summary: RapiScan vs Blood

Analyte	TOTAL	CANNABIS	AMPHETAMINES	OPIATES	COCAINE	BENZOS
Total n	176	35	35	35	35	35
TP	22	12	1	4	4	1
TN	125	14	27	30	28	26
FP	21	5	7	0	3	6
FN	7	4	0	1	0	2
Sensitivity	75.9 %	75.0 %	100.0 %	80.0 %	100.0 %	33.3 %
Specificity	85.6 %	73.7 %	79.4 %	100.0 %	90.3 %	81.3 %
Positive predictive value	51.2 %	70.6 %	12.5 %	100.0 %	57.1 %	14.3 %
Negative predictive value	94.7 %	77.8 %	100.0 %	96.8 %	100.0 %	92.9 %
Prevalence	16.5 %	45.7 %	2.9 %	14.3 %	11.4 %	8.6 %
Accuracy	84.0 %	74.3 %	80.0 %	97.1 %	91.4 %	77.1 %

Table 8: Results of on-site RapiScan device as compared to results in urine

Summary: RapiScan vs Urine

Analyte	TOTAL	CANNABIS	AMPHETAMINES	OPIATES	COCAINE	BENZOS
Total n	175	35	35	35	35	35
TP	24	16	0	3	4	1
TN	120	10	26	30	28	26
FP	16	1	7	0	2	6
FN	15	8	2	2	1	2
Sensitivity	61.5 %	66.7 %	0.0 %	60.0 %	80.0 %	33.3 %
Specificity	88.2 %	90.9 %	78.8 %	100.0 %	93.3 %	81.3 %
Positive predictive value	60.0 %	94.1 %	0.0 %	100.0 %	66.7 %	14.3 %
Negative predictive value	88.9 %	55.6 %	92.9 %	93.8 %	96.6 %	92.9 %
Prevalence	22.3 %	68.6 %	5.7 %	14.3 %	14.3 %	8.6 %
Accuracy	82.3 %	74.3 %	74.3 %	94.3 %	91.4 %	77.1 %

Comparison of Oral Fluid (Intercept) and Blood Results:

Table 9: Laboratory findings of oral fluid as compared to results in blood

Summary: CHT Oral Fluid vs Blood

Analyte	TOTAL	CANNABIS	AMPHET	METHAMP	COCAINE	OPIATES	BENZOS
Total n	277	47	46	46	46	46	46
TP	46	26	2	2	5	7	4
TN	216	19	42	41	36	39	39
FP	12	0	2	3	5	0	2
FN	3	2	0	0	0	0	1
Sensitivity	93.9 %	92.9 %	100.0 %	100.0 %	100.0 %	100.0 %	80.0 %
Specificity	94.7 %	100.0 %	95.5 %	93.2 %	87.8 %	100.0 %	95.1 %
Positive predictive value	79.3 %	100.0 %	50.0 %	40.0 %	50.0 %	100.0 %	66.7 %
Negative predictive value	98.6 %	90.5 %	100.0 %	100.0 %	100.0 %	100.0 %	97.5 %
Prevalence	17.7 %	59.6 %	4.3 %	4.3 %	10.9 %	15.2 %	10.9 %
Accuracy	94.6 %	95.7 %	95.7 %	93.5 %	89.1 %	100.0 %	93.5 %

Discussion

General: A high refusal rate for subject participation resulted in poor subject recruitment numbers during Phase 1. Subjects often found no reason to cooperate further with the officer who just arrested them. The research was conducted in several counties, none with major cities or a centralized testing facility where a researcher or neutral party that was not involved in the arrest could recruit subjects. In spite of this some officers were quite successful in recruiting subjects and their experiences will be shared and incorporated into Phase 2 procedures.

OraLab: The OraLab device fared poorly under the conditions of this study. Subjects were often unable to provide sufficient oral fluid during specimen collection, resulting in many invalid tests. The high rate of invalid tests influenced some officers to lose enthusiasm for the study and stop recruiting subjects. Officers also experienced difficulty observing the presence or absence of the test lines making interpretation of results inconsistent.

RapiScan: The RapiScan is a hand held instrumented device. The operating procedure of the instrument is fairly direct, but was found to intimidate officers if they were not able to use it soon after training. Many officers indicated they were uncomfortable using the instrument, stating that it was difficult to remember the procedure and that they would have preferred to use a non-instrumented or manually interpreted device.

Oral Fluid vs. Blood: Data from blood and oral fluid specimens tested in the laboratory were collected in 46 cases (47 for the cannabinoids test). A comparison of these data demonstrates good correlation, confirming previous findings that validate oral fluid as a suitable specimen for drug testing purposes.

Conclusions

Detailed conclusions will be forthcoming at the conclusion of the Phase II data collection period in the final ROSITA 2 US Sites Report.

Acknowledgements

The authors would like to express their gratitude to the officer-researchers and their agencies for their outstanding support with this project. Special recognition is extended to Corporal Paul Nell for his assistance with officer training and for his prolific subject recruitment activities.

The authors also wish to recognize the significant support provided by the WSLH Forensic Toxicology Program personnel, with special thanks to William Johnson, Kristin Drewieck and Amy Cochems for their expert assistance.

APPENDIX A

Participating Police Agencies, Officers and Device Assignment

Brown County (OraLab)

Brown County Sheriff's Office

Trevor Bilgo
Derrick R. Brown
Kevin J. Kinnard
Karl R. Lau
Guy Shepardson
Brian Westphal

DePere Police Department

Zachary Roush
Nathan G. Thompson

Dodge County (RapiScan)

Dodge County Sheriff's Office

Paul Nell
Theodore Sullivan

LaCrosse County (RapiScan)

LaCrosse County Sheriff's Office

Richard G. Amundsen
Andrew N. Dittman
William Lubinski
Brandon J. Stoughtenger
John P. Williams
Erich Zwicher

Manitowoc County (OraLab):

Manitowoc County Sheriff's Office

Andrew L. Colborn
Todd A. Cummings
Joseph E. Keil
Michael A. Polich

Manitowoc Police Department

Jennifer M. Kneeland
Jason P. Koenig
Scott S. Luchterhand
Larry Perronne
Nick Reimer
Michael A. Stone
Joan T. Waskow

Two Rivers Police Department (OraLab)

Melissa Becker
Timothy M. Culligan
Shawn Engleman
Robert F. Kappelman
Karen Schleis
Jeremy J. Stodola

Marathon County (OraLab)

Rothschild Police Department

Ronald Dallman
Jason Foemmel
Colleen A. Gertschen
Jeremy P. Hunt

Wausau Police Department

Matthew Barnes
Benjamin K. Bliven
Dwayne J. Dachel
Thomas J. Hines

Waukesha County (OraLab)

New Berlin Police Department

Chris Jaekl
Brian B. O'Keefe

Waukesha County Sheriff's Office

Neil S. Dussault
Michael E. Goldmann

APPENDIX B RESEARCH INFORMATION AND INSTRUCTION

TITLE OF STUDY: ROSITA 2 – Evaluation of Oral Fluid (Saliva) Drug Detection Devices

You are invited to be in a research study to test new drug detection devices that use saliva. To be in the study, you need to provide saliva and urine specimens as follows:

We will be collecting two separate saliva specimens. For each specimen you will need to place a sterile pad inside your mouth for one to two minutes. After two minutes the pad will be removed from your mouth.

If you choose to participate and provide saliva specimens:

- ✓ The specimens will be used for research purposes only.
- ✓ The results will be kept completely private.
- ✓ Your name will not be placed on any research specimen or form. All research specimens and forms will be labeled only with a case number.
- ✓ All information that may tie your name to the results will be removed after all testing is completed (3 months time at most). Therefore, after that is done you cannot be connected to the results.
- ✓ The saliva specimens will be discarded in a confidential manner after all testing is completed.
- ✓ Everyone who handles the data and information from the saliva specimens will sign a confidentiality certificate that requires them to not reveal any of the information.
- ✓ There are no known risks to your being in the study.
- ✓ There will be no discomfort in placing the collection devices in your mouth.
- ✓ The saliva collection devices are sterile and the person collecting the saliva has been trained to do so in a manner that is safe.
- ✓ The results of your saliva and urine tests **cannot be used against you** in court. There is a letter from the District Attorney of this county that promises they will not use the research results in any legal proceedings.
- ✓ Your participation is completely voluntary.
- ✓ Nothing will happen to you if you choose not to be in this study.
- ✓ You may stop and quit the study **at any time** without penalty.
- ✓ There is NO benefit to you as a result of being in this study.
- ✓ Taking part in the study will not impact your case in a good or bad way.

This project is being funded by the Office of National Drug Control Policy (ONDCP), The National Institute on Drug Abuse (NIDA) and The National Highway Traffic Safety Administration (NHTSA) under the direction of The Walsh Group, P.A. If you have any questions about the research project, the risks involved, and/or your rights as a participant, you may contact us at the following number / address:

UWHC Patient Relations Representative
Tel: (608) 263-8009

William C. Sonzogni, PI
Laura J. Liddicoat, Coordinator
2601 Agriculture Drive
Madison, WI 53718
Tel: (608) 224-6241

J. Michael Walsh, Ph.D., P.I.
6701 Democracy Blvd. Ste 300
Bethesda, MD 20817
Tel: 1-800-985-1225

Revision Date: February 10, 2004

Cozart® RapiScan 5-Panel Drug Test
(Opiate/Cocaine/Amphetamine/Benzodiazepines/Cannabis)

Catalogue No.:	CZR511
Sample Type:	Oral Fluid (Saliva)
Description:	Each box contains 25 test cartridges in sealed laminate foil pouches which will detect the following groups of abused drugs in oral fluid in 12 minutes.
Sample preparation:	Collect oral fluid sample using the Cozart® Collection pad as provided within the test pack.
Shelf Life:	18 months from manufacture.

Specificity

A broad range of related and non-related compounds have been tested:

Non cross reacting compounds have been tested at a concentration of 10,000 ng/mL. The Cozart RapiScan response was OPI, COC, AMP, BZO and THC negative for all of these compounds.

Amitriptyline	LSD
Acetyl Salicylic Acid (Aspirin)	Methadone
Acetaminophen (paracetamol)	Methylphenidate
Amobarbital	Nicotine
Ascorbic Acid	Paracetamol
Buprenorphine	Phenylpropanolamine
Caffeine	Phenobarbital
Cotinine	DL-Propranolol
Chloroquine	Ranitidine
Dextromethorphan	Quinalbarbitone
Gentamycin	

Cross-reactants

Compound	ng/mL	Cozart® RapiScan Response
D Amphetamine	15	AMP Positive +ve
MDA (ADAM)	10	AMP Positive +ve
D Methamphetamine	10,000	AMP Positive +ve
MDMA (ECSTASY)	10,000	AMP Positive +ve
MBDB	10,000	AMP Negative -ve
MDEA (EVE)	10,000	AMP Negative -ve
(+) Ephedrine	10,000	AMP Negative -ve
(-) Ephedrine	10,000	AMP Negative -ve
Fenfluramine	10,000	AMP Negative -ve
Tyramine	10,000	AMP Positive -ve
Temazepam	5	BZO Positive +ve
Oxazepam	10	BZO Positive +ve
Diazepam	10	BZO Positive +ve
Desmethyldiazepam	10	BZO Positive +ve
Alprazolam	10	BZO Positive +ve

Cross Reactants Continued

Compound	ng/mL	Cozart® RapiScan Response
Nitrazepam	5	BZO Positive +ve
Prazepam	10,000	BZO Positive +ve
Triazolam	25	BZO Positive +ve
Lorazepam	100	BZO Positive +ve
Midazolam	250	BZO Positive +ve
Flunitrazepam	10	BZO Positive +ve
Clobazam	10	BZO Positive +ve
7 Amino-Flunitrazepam	10,000	BZO Negative -ve
Flurazepam	10,000	BZO Negative -ve
Cocaine	30	COC Positive +ve
Benzoyllecgonine	10	COC Negative -ve
Cocaethylene	10	COC Positive +ve
Lidocaine	10,000	COC Negative -ve
Ecognine Methyl Ester	10,000	COC Negative -ve
Δ 9THC	50	THC Positive +ve
11-OH-THC Δ 9THC	25	THC Positive +ve
Cannabidiol	100,000	THC Negative -ve
Morphine	10	OPI Positive +ve
6 Monoacetylmorphine	10	OPI Positive +ve
Heroin (Diacetylmorphine)	20	OPI Positive +ve
Dihydrocodeine	10	OPI Positive +ve
Codeine	10	OPI Positive +ve
Morphine-3- glucuronide	20	OPI Positive +ve
Pholcodeine	10	OPI Positive +ve
Nalorphine	20	OPI Positive +ve

For Cocaine, Morphine, Amphetamine, Methamphetamine, PCP, and Cannabinoids

Part No. 6902004

English

Intended Use

On-Site OraLab® detects drugs of abuse in saliva and provides for the collection, transport, and storage of saliva specimens. OraLab is intended for forensic use in simultaneous, qualitative detection of multiple drugs and metabolites in saliva. The OraLab profile is based, when possible, on the preliminary oral fluid testing requirements of the U.S. Federal Workplace Drug Testing Programs.¹

OraLab Cutoff Concentrations

		ng/mL
COC	Cocaine	20
MOR	Morphine	40
AMP	<i>d</i> -Amphetamine	50
MET	<i>d</i> -Methamphetamine	50
PCP	Phencyclidine	10
THC	Δ^9 -Tetrahydrocannabinol	100

OraLab provides only preliminary analytical test results. An alternate, more specific chemical method must be used to obtain confirmed analytical results. Mass spectrometry (GC/MS or LC/MS/MS) is the preferred confirmation method for saliva specimens.¹ Clinical consideration and professional judgment should be applied to drugs of abuse test results, particularly preliminary positive results.

Summary

Drugs may be ingested, inhaled, or injected.² After entering the bloodstream, they are rapidly metabolized by various pathways.³ Many drugs and drug metabolites are excreted in urine and detected by traditional drug urine screening assays. Alternative laboratory methods have been used to detect drugs in blood or serum.

The utility of saliva as a biological specimen for the detection of recent drug use has been supported by a number of studies.⁴⁻⁸ A growing body of literature describes the detection and related pharmacokinetics of cocaine, opiates, amphetamines, phencyclidine, and cannabinoids in saliva.^{8,9} Correlation of drug in saliva and blood has been reported.¹⁰⁻¹³ Unlike urine drug tests, saliva drug testing detects active drugs present at low concentrations (recent drug use, typically within hours).

Historically, drug-screening assays relied on classical chemical methods, such as thin-layer chromatography or liquid chromatography, which are accurate but laborious procedures. More recent assays for initial testing are based on immunoassay principles. Examples of these methods are radioimmunoassay, enzyme immunoassay, kinetic interaction of microparticles in solution, and fluorescence immunoassay. These methods require sophisticated instrumentation and the handling of laboratory reagents and urine specimens. The classic drug screening technology incorporated in OraLab provides the accuracy of immunoassays but does not require the collector to witness urine collection or handle laboratory reagents and urine specimens.

OraLab is a simple, qualitative, visually read 10-minute test that detects recent drug use using saliva specimens. The design permits convenient collection and lateral flow screening, with a split specimen stored in a tamper-evident container for confirmation testing.

Test Principle

OraLab assays are based on the principle of competitive inhibition. Drug that may be present in the saliva competes with drug conjugate immobilized on the test membrane for binding sites on the antibody-coated microparticles. A visual signal is generated through classical lateral flow technology.

Specimen Collection: Saliva is collected by absorption directly into the OraLab foam-tipped saliva collector and is delivered into the test cassette

by slowly pushing the collector foam into the sample well on the OraLab cassette. A portion of the saliva specimen contacts reagents and initiates the screening test.



(Figure 1)

The Test: The saliva interacts with antibody-coated microparticles and drug conjugate present on the membrane. In the absence of drug, the antibody is free to react with the drug conjugate, causing the formation of a red/pink band in each drug zone. When drug is present in the specimen, it binds to the antibody-coated microparticles. If sufficient drug is present, the microparticles are inhibited from binding the drug conjugate, and no band is formed. A preliminary positive ("non-negative") specimen causes the detection area on the membrane to remain blank.

Quality Control: An additional antibody/antigen reaction is designed into the Test Valid area. When the specimen application and procedure are appropriate, antibodies on the reagent membrane bind the antigen on the microparticles, and the Test Valid band forms.

Chain of Custody: Excess saliva collects in the confirmation well, which is sealed with the cap and tamper evident tape, if required. The cassette is ready for delivery to a confirmation site for laboratory testing.

Precautions & Warnings

OraLab test cassettes are for forensic use in testing human saliva for designated drugs.

Specimens containing human-sourced materials should be handled as if potentially infectious. Use safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number [CDC] 93-8395).

Storage & Stability

Store OraLab cassettes at room temperature, 15 - 30 °C (59 - 86 °F), in the original foil pouch. If the foil pouch containing the OraLab test cassette is damaged (e.g., a hole or tear), do not use that OraLab cassette. Do not use OraLab cassettes after the expiration date on the foil pouch.

Collection & Testing

Reagents & Materials Provided

1. An OraLab foil pouch contains:

- One OraLab saliva collector sealed in a bag
- One OraLab cassette, containing:

A sample well that receives saliva from the collector and delivers it to the screening reagents and the confirmation well.

Two individual test chambers for detection of multiple drugs or drug metabolites. The reagent strip in each test chamber contains:

- Microparticles coated with antibodies reactive with the drugs in a buffered solution containing preservative dried on a release pad
- Drug or drug analogue conjugates immobilized on a membrane
- Antibody reactive to mouse immunoglobulin immobilized on a membrane
- Absorbent pads used to filter the specimen and regulate the flow of liquids within the cassette
- Confirmation well and cap

c. Desiccant pouch

2. Instruction card and tamper-evident labels

3. Biohazard bags for shipment to confirmation site or device disposal, if required

Ancillary Materials

	Part No.	Quantity
Latex Gloves	42210	50 pairs
Collection Cups	47123	100 cups/box

Collect Saliva & Start the Test

Before the test is administered, instruct the donor to drink four ounces of water, and to place nothing else in the mouth such as food, drink, gum, or tobacco products for 10 minutes prior to collection. During the minute or two prior to collection, instruct the donor to draw a pool of saliva into the mouth with a "puckering" action.

Record the donor name and collection date on the cassette.

Collect fresh saliva specimens as follows; no preservative, special handling, or pretreatment is required

1. Instruct the donor to open the collector bag, place the collector foam inside mouth for 3 minutes, completely filling the collector foam with saliva.

Specifically, instruct that the donor:

- Bathe the collector foam in the pool of saliva
- Alternatively, stimulate salivation by continuously moving the collection foam along the sides and lower margin of the tongue
- Not chew, compress, or suck on the collector foam

2. Observe the collection.

3. Place the OraLab cassette on a flat surface and lift the confirmation well cap to assure that the flow of saliva into the well is not obstructed.

4. Remove the collection foam from the mouth. It should be very wet when removed from the mouth, and must not be compressed when removed.

- Place the collector foam into the sample well on top of the device, and gently but firmly push the collector slowly (approximately five seconds) downward until it comes to a firm stop.
- Saliva flows directly into the test cassette.

Interpret Test Validity and Results

Test validity and results must be interpreted between 10 and 15 minutes after initiating the test.

Seal the confirmation well 10 minutes after initiating the test by pushing the cap down. The "click" confirms that the well is properly sealed.

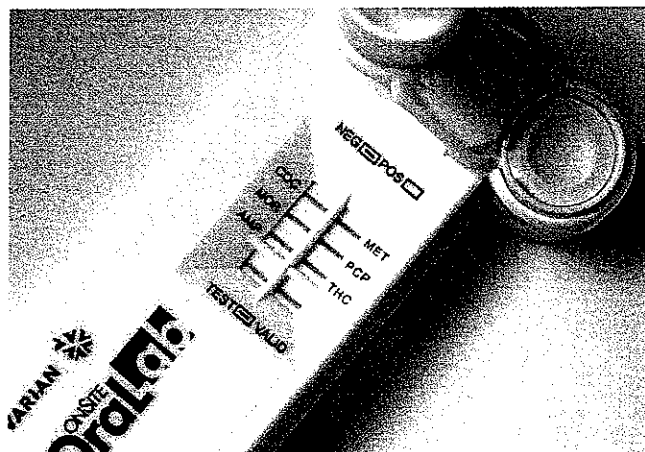


(Figure 2) Remove the label covering the read windows

1. Interpret test validity: A valid test is indicated by a band in the Test Valid window. Both of the COC/OPI/AMP and MET/PCP/THC reagent strips pictured below (Figure 3) are valid. Since a valid test may give a faint or incomplete band, any Test Valid band confirms that the test is valid. Due to the high viscosity and variability of some saliva samples, test results may require up to 15 minutes to form. An invalid test is indicated by the absence of a distinct band in the Test Valid window or by a reddish reagent background in the Results or Test Valid windows which obscures the presence of bands 15 minutes after the test is initiated. If an invalid result is obtained, see Step 3 below.

2. Immediately interpret test results as either negative or preliminary positive.

- a. A negative result for a given drug (i.e., drug absent or below the cutoff) is the presence of a band in the Results window adjacent to the drug name. The intensity of the bands in the Results windows may vary. A negative sample may give a faint or incomplete band; any band in the Result window indicates a negative result. The test results pictured below (Figure 3) are negative for Cocaine, Morphine, Amphetamine, Methamphetamine, PCP, and THC.



(Figure 3)

- b. A preliminary positive result for a given drug (i.e., drug present above the cutoff, suggesting current drug use) is the absence of a band in the test result window adjacent to the drug name. The adjacent area appears off-white.

c. If all drug results are negative, or if no further confirmation tests are required, properly discard the test cassette and collector.

d. When confirmation of a preliminary positive screen result is required:

- Verify that the volume of saliva in the confirmation well is sufficient for laboratory testing by observing the transparent bottom of the confirmation well. A sufficient specimen will fill at least half of the well.
- Observe any chain of custody procedures in effect at the collection site. Complete chain of custody paperwork as required.
- Have the donor initial and date the tamper-evident tape. Apply the tape securely over the cap (Figure 4).
- Seal the cassette in the bag provided.
- Send the specimen and paperwork to the confirmation site.



(Figure 4)

3. If test did not run, if invalid test results are obtained, or if the confirmation well is less than half full, **do not add additional sample**. Use a second OraLab device, after discarding the first.
 - a. Instruct the donor to drink 4 ounces of water. Assure that this water is swallowed and that nothing else is placed in the mouth for three to five minutes.
 - b. During the minute or two prior to collection, instruct the donor to draw a pool of saliva into the mouth with a "puckering" action.
 - c. Provide a second packaged collector to the donor, and repeat collection instructions with emphasis on:
 - Bathing the collection foam in saliva, and/or
 - Continuous gentle movement of the collector over the tongue throughout the collection
 - d. Observe a **4-minute** collection and perform the test according to the instructions in "Collect Saliva & Start Test" section.
 - e. Remove and properly discard the OraLab saliva collector

Quality Control

Quality control testing at regular intervals is good analytical practice and may be required by federal, state, or local guidelines. Always check with the appropriate licensing or accrediting bodies to ensure your quality control program meets the established standards.

Internal control: Each OraLab cassette has a built-in process control. The Test Valid band should always appear if the test procedure is performed properly and the specimen is adequate. In addition, the red background of reagents in the read windows normally clears to off-white, providing a distinct result 10 minutes after initiating the test. A Test Valid band is an internal process control. Partial, incomplete, or broken bands still indicate a valid test.

External control: The use of positive and negative saliva controls is recommended to test each shipment of product, when a new lot is used, or anytime the product has been stored outside the recommended storage conditions.

Contact Varian, Inc., for information regarding commercially available controls. These controls should produce the expected results. If these results are not obtained, call Technical Support for assistance.

Limitations

1. Rare false positive or false negative results may occur as a result of nonspecific interactions (physiological variation, state of health, contamination of saliva with food or other substances, etc.)
2. Antibodies used in these reagents are very specific for targeted drugs of abuse. Nevertheless, structurally similar prescribed and over-the-counter drugs can react with the antibody reagents and cause false positive results. A more specific alternate chemical method such as GC/MS or LC/MS/MS must be used to obtain a confirmed analytical result. A test protocol for simultaneous GC/MS determination of these drugs in saliva is available from Varian, Inc., for validation and use by commercial laboratories; contact Varian, Inc., for further information. Clinical consideration and professional judgment should be applied to any drug abuse test result, particularly preliminary positive test results, and should be reviewed by the Medical Review Officer.
3. Saliva collection may be closely observed. As a result, specimen adulteration is unlikely. Nevertheless, if adulteration or substituted sample is suspected, discard that specimen and collect a second specimen for testing.

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APPENDIX E

CASE NO: _____

OFFICER ID: _____

ROSITA2 DATA COLLECTION FORM

POLICE OFFICER INFORMATION

Time of Stop: _____

Site Location: Wisconsin B

Reason For Drug Test:

- ☐ Unusual appearance, behavior or odor indicating drug use
- ☐ Failed SFST and BAC level < 0.08
- ☐ Had drug / drug paraphernalia in vehicle
- ☐ BAC level inconsistent with impairment
- ☐ Driver admits to using drugs. Which drug(s)? _____

Breath AC Test Results:

Type Test	BrAC	Time
PBT / Evidentiary (Circle One)		

Saliva Rapid Drug Test Device: RapiScan

Indicate Positive Result with (+) and Negative with (-) for each drug

	BZO	AMP	THC	COC	OPI
Test Results					

Specimen Collection Time: Oral Fluid (Test): _____ Oral Fluid (For Lab): _____ Urine: _____

*DID SUBJECT VOMIT DURING SALIVA COLLECTION? YES NO (CIRCLE ONE)

Officers Comments: _____

FOR LABORATORY USE ONLY

LABORATORY ACCN #: _____

Indicate Positive with (+) and Negative with (-) and quantitative levels (ng/mL)

Drug	Confirmation Analyte	Oral Fluid		Blood		Urine	
		Screen	Confirm	Screen	Confirm	Screen	Confirm
AMP	AMP / METH	(+) or (-)	LOD	(+) or (-)	LOD	(+) or (-)	LOD
	Amphetamine						
METH	Methamphetamine						
	MDMA (Ecstasy)						
	MDEA						
COC	COC						
	Cocaine						
	Benzoyllecgonine						
THC	THC						
	9THC (parent)						
	THCCOOH (metab.)						
OPI	OPI						
	Morphine						
	Codeine						
	Hydromorphone						
	Oxycodone						
BDP	Hydrocodone						
	BDP						
	Diazepam						
	Oxazepam						
	Others:						

Comments: _____

APPENDIX F

CASE NO: _____

OFFICER ID: _____

ROSITA2 DATA COLLECTION FORM

POLICE OFFICER INFORMATION

Time of Stop: _____

Site Location: Wisconsin A

Reason For Drug Test:

- ☐ Unusual appearance, behavior or odor indicating drug use
- ☐ Failed SFST and BAC level < 0.08
- ☐ Had drug / drug paraphernalia in vehicle
- ☐ BAC level inconsistent with impairment
- ☐ Driver admits to using drugs. Which drug(s)? _____

Breath AC Test Results:

Type Test	BrAC	Time
PBT / Evidentiary (Circle One)		

Saliva Rapid Drug Test Device: OraLab

Indicate Positive Result with (+) and Negative with (-) for each drug

	COC	MOR	AMP	MET	PCP	THC
Test Results						

Specimen Collection Time: Oral Fluid (Test): _____ Oral Fluid (For Lab): _____ Urine: _____

*DID SUBJECT VOMIT DURING SALIVA COLLECTION? YES NO (circle one)

Officers Comments: _____

FOR LABORATORY USE ONLY

LABORATORY ACCN #: _____

Indicate Positive with (+) and Negative with (-) and quantitative levels (ng/mL)

Drug	Confirmation Analyte	Oral Fluid		Blood		Urine	
		Screen	Confirm	Screen	Confirm	Screen	Confirm
AMP	AMP / METH	(+) or (-)	LOD	(+) or (-)	LOD	(+) or (-)	LOD
	Amphetamine						
METH	Methamphetamine						
	MDMA (Ecstasy)						
	MDEA						
COC	COC						
	Cocaine						
	Benzoyllecgonine						
THC	THC						
	9THC (parent)						
	THCCOOH (metab.)						
OPI	OPI						
	Morphine						
	Codeine						
	Hydromorphone						
	Oxycodone						
BDP	Hydrocodone						
	BDP						
	Diazepam						
	Oxazepam						
	Others:						

Comments: _____

APPENDIX G

Intercept Oral Fluid Collection Procedures



Step 1

Inspect the expiration date on the Intercept® collection device packaging and open the outer packaging containing the collection pad and the specimen vial. Place the specimen vial on a flat surface in view of the donor. Instruct the donor to remove the handle and remove the collection device from the packaging sleeve.



Step 2

Instruct the donor to place the collection pad between the lower cheek and gums and gently rub the pad back and forth along the gum line until the pad is moist.



Step 3

Once moist, leave collection pad between cheek and gums for a full two (2) minutes.



Step 4

After 2 minutes, have the donor open the specimen vial in an upright position, with the tip pointed downward, by gently rocking the cap back and forth to avoid spilling the contents. Instruct the donor to push the collection pad into the specimen vial as far as it will go.



Step 5

Instruct the donor to snap the collection wand at the scored line against the side of the vial. Instruct the donor to not tilt the vial or spill the fluid inside.



Step 6

Instruct the donor to place the cap onto the vial until it snaps, ensuring a secure fit.



Step 7

Instruct donor to place tamper evident seal across the top of the specimen vial and down the sides, date and initial.

Chapter 13. Evaluation of rapid Point-of-collection oral fluid testing devices

Elke Raes, Alain Verstraete

Department of Clinical Biology, Microbiology and Immunology
Ghent University

Abstract

The Rosita-2 study evaluated rapid point-of-collection oral fluid drug testing devices at the roadside or in a police station. The number of included subjects was 2046 and 2605 device evaluations were performed. Nine devices were evaluated: American Biomedica Oralstat, Branam Medical Oratect, Cozart Bioscience RapiScan (only in the USA), Dräger/Orasure DrugTest/Uplink, Lifepoint Impact, Securetec Drugwipe, Sun Biomedical Oraline, Ultimed SalivaScreen and Varian OraLab.

For six devices (Varian Oralab, Lifepoint Impact, Branam Oratect 2nd generation, Sun Oraline, Ultimed SalivaScreen and Branam Oratect), more than 25% of the devices failed to run. For the other devices, the number of failures was less than 10% (American Biomedica Oralstat and Dräger DrugTest/Orasure Uplink) or less than 5% (Cozart RapiScan and Securetec Drugwipe).

The percentages of positive samples were: amphetamines (including methamphetamine, ecstasy and analogues) 20%, benzodiazepines 32%, cannabinoids 31%, cocaine 19% and opiates 8%.

The analytical evaluation of the **amphetamine and methamphetamine** tests (in comparison to the reference method in oral fluid) showed a sensitivity varying between 40% and 83% and a specificity between 80% and 100%. The analytical evaluation of the **benzodiazepine** tests showed a sensitivity varying between 33% and 69% and a specificity between 85% and 94%. The analytical evaluation of the **cannabis** tests showed a sensitivity varying between 0% and 74 % and a specificity between 70% and 100%. Detailed analysis of the data for cannabis showed that some devices (e.g. Drugwipe) gave a negative result even when very high concentrations of THC were found with the Intercept. The analytical evaluation of the **cocaine** tests showed a sensitivity varying between 0% and 97% and a specificity between 91% and 100%. The analytical evaluation of the **opiate** tests showed a sensitivity varying between 51% and 100% and a specificity between 86% and 100%.

No device met the criteria proposed during the Rosita-1 project (sensitivity and specificity > 90%, accuracy > 95%) for the amphetamines, benzodiazepines and cannabis. The Varian Oralab met these criteria for cocaine and opiates, but it gave 26% failures, so it cannot be recommended.

The operational evaluation of the devices showed that the sampling technique was well accepted by the police and the subjects for Drugwipe, Dräger DrugTest and Sun Biomedical Oraline. For the Oralstat, Oratect and Varian OraLab there were problems.

For some devices, e.g. cannabis on the Drugwipe or Sun Biomedical Oraline, the results were difficult to read. Several devices had problems when used in cold and rainy weather.

Police officers preferred a system that reads the result automatically, but the device should also be readable when no instrument is available.

The procedure for some devices, e.g. Dräger DrugTest and Cozart Bioscience RapiScan was considered too lengthy and complicated. The high number of device failures had a negative effect on the motivation of the police officers.

At the end of the study, no device was considered to be reliable enough in order to be recommended for roadside screening of drivers.

Introduction

The result of the evaluation in the different countries and states is given in chapters 3-12. This chapter will give the results of the evaluation of the aggregated data. The following aspects will be discussed: number of device failures, analytical evaluation of the onsite devices, comparison of the results of the lab analyses on oral fluid and blood in order to estimate the best cut-off, and the evaluation of the operational aspects of the use of the 9 onsite oral fluid devices. Finally, the impact of the Rosita-2 study on national legislation and law enforcement will be given.

Device failures

During the course of the study, an increasing number of reports were made on frequent failure of the devices. Depending on the type of device, this was apparently due to too little or too viscous saliva (the fluid didn't migrate until the control line, or it caused smears), or to a malfunctioning of the instrument that reads the results.

Table 1 gives an overview of the number of devices which failed to give a result.

Table 1: Percentage of device failures, in increasing order.

Device	Failed	Total	%
Cozart RapiScan	0	40	0%
Securetec Drugwipe	50	1364	4%
American Biomedica Oralstat	3	52	6%
Dräger DrugTest	52	592	9%
Varian Oralab	61	234	26%
Lifepoint Impact	14	44	32%
Branan Oratect II	20	53	38%
Sun Oraline	15	38	39%
Ultimed SalivaScreen	33	70	47%
Branan Oratect	87	118	74%
Total	335	2605	13%

During the meeting in Santiago de Compostela in November 2004, the problem of failures and the resulting disappointment and decreasing motivation of the police officers were discussed. It was felt that a device should have a maximum of 5% failures (10% at the very maximum). Based on this criterion, only Cozart RapiScan and Securetec Drugwipe would be acceptable. It is perhaps not surprising that both companies have the longest experience in onsite oral fluid testing. American Biomedica Oralstat and Dräger DrugTest fall within the 10% criterion.

One could argue that the percentage of failures is related to the training of the users and that the high percentages are explained by poor training in some countries. However, table 2 shows that (for the devices where a comparison is possible, the percentage of failures is remarkably similar, e.g. for Drugwipe, it is never higher than 8%, while for Oratect and Oralab it is always higher than 52% and 25% respectively.

Table 2: percentage of device failures in the different countries.

	Germany	Norway	Spain	Belgium	France	Finland	USA
Drugwipe		8%		1%	7%	5%	1%
DrugTest	10%	18%	16%	4%			4%
Oratect	86%	67%					52%
Oratect II	38%						
Impact	32%						
Oralstat	6%						
Oraline		39%					
Oralab			25%	80%			29%
RapiScan							0%
SalivaScreen							47%

Analytical evaluation of the onsite devices

Methods

The analytical methods used in the ten collaborating centres are described in chapter 3 to 12. The data from the evaluations in the ten collaborating centres were obtained in Microsoft Excel format. For the overall evaluation of the results, the individual files were joined and the cut-offs in table 3 were used for the determination of a positive/negative in neat oral fluid or in blood.

Table 3: Cut-offs used for the overall analysis.

Substance	LOQ blood (ng/mL)	LOQ oral fluid (ng/mL)*
Amphetamine	20	25
Methamphetamine	20	25
MDMA	20	25
MDA	20	25
MDEA	20	25
Cocaine	20	8
Benzoyllecgonine (BE)	20	8
Morphine	10	20
Codeine	10	20
THC	1	2
THCCOOH	5	
11-OH-THC	1	
Diazepam	50	5
Nordiazepam	50	5
Temazepam	50	5
7-aminoflunitrazepam	1	2
Bromazepam	10	5
Lorazepam	10	5
Clonazepam	5	5
(Zopiclone/Zolpidem)	10	10

*: neat oral fluid

The evaluation of the results was based on classification into the following categories:

- **TN:** True Negatives: number of samples in which screening was negative or analyte

- concentrations are below GC-MS cut-off values and correctly classified by the test.
- **TP:** True Positives: number of samples in which analyte concentrations are above GC-MS cut-off and classified correctly by the test.
 - **FP:** False Positives: number of samples misclassified as a positive result by the screening test, that was not confirmed by GC-MS to contain analytes at concentrations above the cut-off values.
 - **FN:** False Negatives: number of samples giving a negative result with the screening test but in which analytes are present at concentrations above the GC-MS cut-off values.

From these 4 categories, the following characteristics were calculated:

- **Sensitivity:** true positives expressed as percent of all positives = $TP/(TP+FN)$
- **Specificity:** true negatives expressed as percent of all negatives = $TN/(TN+FP)$
- **Positive Predictive Value (PPV):** percent of samples with positive results who are true positives = $TP/(TP+FP)$
- **Negative Predictive Value (NPV):** percent of samples with negative results who are true negatives = $TN/(TN+FN)$
- **Accuracy:** percent of all samples correctly classified by the tests = $(TP+TN)/(\text{all results})$

Analytical evaluation for amphetamines

Criteria

All subjects were classified as being positive or negative for amphetamines and/or XTC, according to the following criteria:

Oral fluid: positive if amphetamine, methamphetamine, MDMA, MDEA or MDA were identified and quantified at or above the cut-offs mentioned in Table 3.

Blood: positive if amphetamine, methamphetamine, MDMA, MDEA or MDA were identified and quantified at or above the cut-offs mentioned in Table 3.

Results

The performance of the different oral fluid testing devices compared with the results of the confirmation in oral fluid is given in Table 4.

Table 4: Oral fluid devices versus confirmation in oral fluid for amphetamines.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	1668	726	187	22	44	29	40	37	583
Prevalence	20.5%	33.7%	1.6%	31.8%	54.5%	13.8%	12.5%	24.3%	7.7%
TP	271	204	2	5	19	3	2	7	29
TN	1250	463	181	15	19	25	28	28	491
FP	76	18	3	0	1	0	7	0	47
FN	71	41	1	2	5	1	3	2	16
Sensitivity	79.2%	83.3%	66.7%	71.4%	79.2%	75.0%	40.0%	77.8%	64.4%
Specificity	94.3%	96.3%	98.4%	100.0%	95.0%	100.0%	80.0%	100.0%	91.3%
Accuracy	91.2%	91.9%	97.9%	90.9%	86.4%	96.6%	75.0%	94.6%	89.2%
PPV	78.1%	91.9%	40.0%	100.0%	95.0%	100.0%	22.2%	100.0%	38.2%
NPV	94.6%	91.9%	99.5%	88.2%	79.2%	96.2%	90.3%	93.3%	96.8%

The performance of the different oral fluid devices compared with the results of the confirmation in blood is given in Table 5.

Table 5: Oral fluid devices versus confirmation in blood for amphetamines.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	743	445	8	4	35	19	35	35	162
Prevalence	45.5%	61.8%	0.0%	25.0%	45.7%	31.6%	2.9%	20.0%	19.8%
TP	300	258	0	1	13	5	1	7	15
TN	359	162	7	3	18	13	27	28	101
FP	29	8	1	0	1	0	7	0	12
FN	38	17	0	0	3	1	0	0	17
Sensitivity	88.8%	93.8%	/	100.0%	81.3%	83.3%	100.0%	100.0%	46.9%
Specificity	92.5%	95.3%	87.5%	100.0%	94.7%	100.0%	79.4%	100.0%	89.4%
Accuracy	90.8%	94.4%	87.5%	100.0%	88.6%	94.7%	80.0%	100.0%	80.0%
PPV	91.2%	97.0%	0.0%	100.0%	92.9%	100.0%	12.5%	100.0%	55.6%
NPV	90.4%	90.5%	100.0%	100.0%	85.7%	92.9%	100.0%	100.0%	85.6%

The Box and Whisker Plot for the amphetamines results of all devices compared to the sum of the concentrations of amphetamines in oral fluid is given in Figure 1. In order to get a better picture of the data, all sums of concentrations higher than 10 000 ng/mL were equated to 10 000 ng/mL.

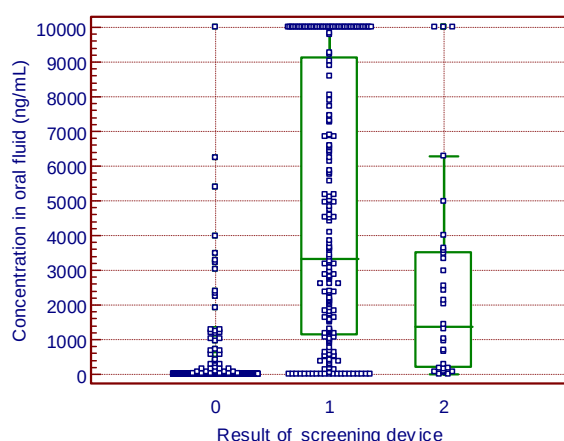


Figure 1: Distribution of the sum of the concentrations of the amphetamines (ng/mL) according to the result of the onsite test (0: negative, 1: amphetamine or methamphetamine test positive, 2: amphetamine and methamphetamine test positive).

The Box and Whisker Plots for the devices separately is given in Figure 2. All sums of concentrations higher than 10 000 ng/mL were equated to 10 000 ng/mL.

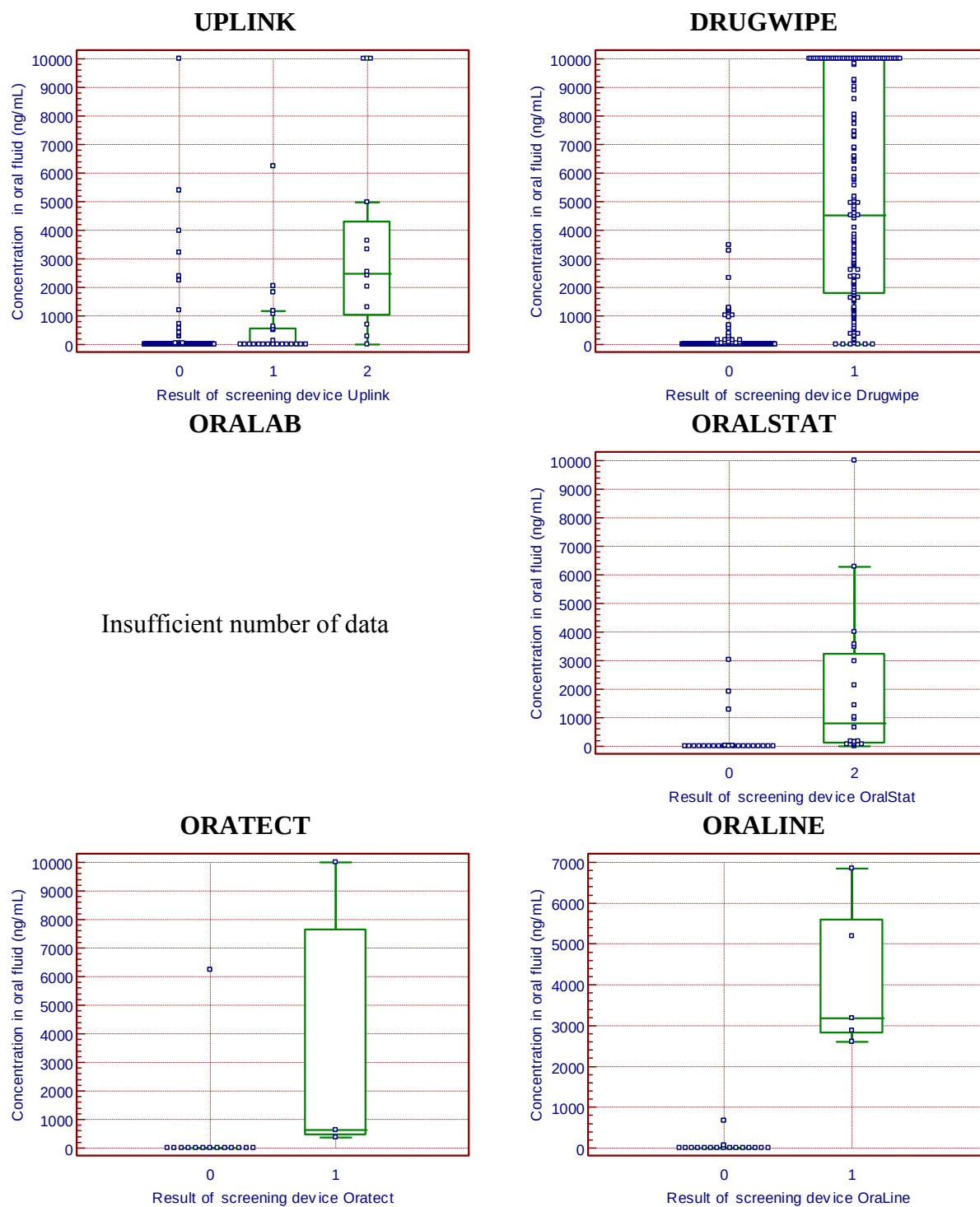


Figure 2: Distribution of the sum of the concentrations of the amphetamines (ng/mL) according to the result of the onsite test (0: negative, 1: amphetamine or methamphetamine test positive, 2: amphetamine and methamphetamine test positive) for the individual devices. All sums of concentrations > 10 000 ng/mL were equated to 10 000 ng/mL.

The cumulative distribution of the sum (> 0 ng/mL) of the concentrations of all amphetamines is given in Figure 3. It illustrates that the concentrations of amphetamines in oral fluid are very high, e.g. 50% of the concentrations are above 2598 ng/mL.

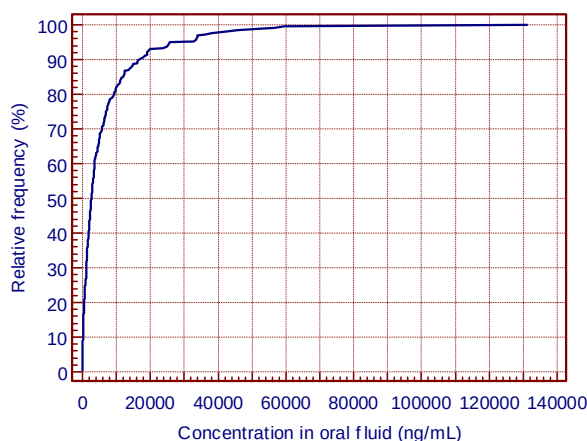


Figure 3: Cumulative distribution of the sum (> 0 ng/mL) of the concentrations of all amphetamines (ng/mL).

Analytical evaluation for benzodiazepines

Criteria

All subjects were classified as being positive or negative for benzodiazepines according to the following criteria:

Oral fluid: positive if a benzodiazepine was identified and quantified at or above the cut-offs mentioned in Table 3

Blood: positive if a benzodiazepine was identified and quantified at or above the cut-offs mentioned in Table 3

Results

The performance of the different oral fluid testing devices compared with the results of the confirmation in oral fluid is given in Table 6.

Table 6: Oral fluid devices versus confirmation in oral fluid for benzodiazepines.

	Total	Drugwipe	RapiScan	SalivaScreen
Total	242	165	40	37
Prevalence	31.8%	41.2%	15.0%	8.1%
TP	51	47	2	2
TN	147	86	29	32
FP	18	11	5	2
FN	26	21	4	1
Sensitivity	66.2%	69.1%	33.3%	66.7%
Specificity	89.1%	88.7%	85.3%	94.1%
Accuracy	81.8%	80.6%	77.5%	91.9%
PPV	73.9%	81.0%	28.6%	50.0%
NPV	85.0%	80.4%	87.9%	97.0%

The performance of the different oral fluid testing devices compared with the results of the confirmation in blood is given in Table 7.

Table 7: Oral fluid devices versus confirmation in blood for benzodiazepines.

	Total	Drugwipe	RapiScan	SalivaScreen
Total	250	180	35	35
Prevalence	35.6%	46.1%	8.6%	8.6%
TP	55	51	1	3
TN	146	89	26	31
FP	15	8	6	1
FN	34	32	2	0
Sensitivity	61.8%	61.4%	33.3%	100.0%
Specificity	90.7%	91.8%	81.3%	96.9%
Accuracy	80.4%	77.8%	77.1%	97.1%
PPV	78.6%	86.4%	14.3%	75.0%
NPV	81.1%	73.6%	92.9%	100.0%

The Box and Whisker Plot for the benzodiazepine results of Drugwipe compared to the sum of the concentrations of benzodiazepines diazepam, nordiazepam, oxazepam, temazepam, alprazolam, clonazepam and lorazepam in oral fluid is given in Figure 4. All sums of concentrations that were higher than 200 ng/mL were equated to 200 ng/mL.

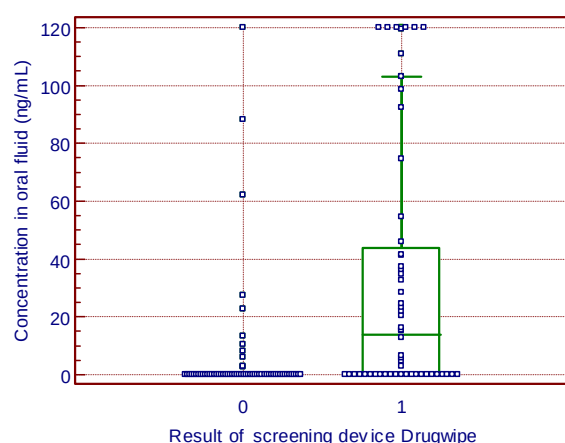


Figure 4: Distribution of the sum of the concentrations of the benzodiazepines according to the result of the onsite test Drugwipe (0: negative, 1: positive). The sums of the concentrations > 200 ng/mL were equated to 200 ng/mL

There were insufficient data to calculate the Box and Whisker Plot for the other devices.

The cumulative distribution of the sum (>0ng/mL) of the concentrations of the benzodiazepines diazepam, nordiazepam, oxazepam, temazepam, alprazolam, clonazepam and lorazepam is given in Figure 5. This figure illustrates that the sum of the benzodiazepine concentrations in oral fluid is low. Fifty % of the concentrations in the positive samples are lower than 25 ng/mL.

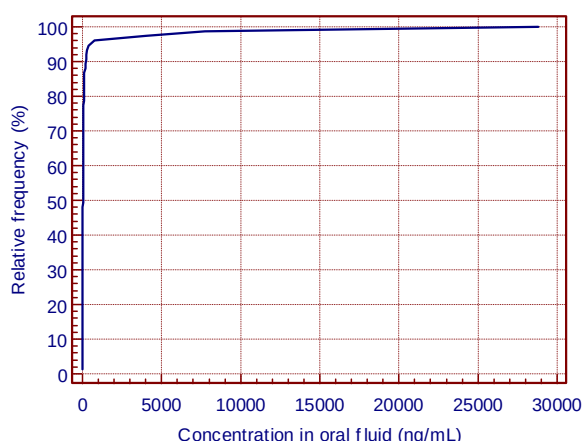


Figure 5: Cumulative distribution of the sum (> 0ng/mL) of the concentrations of benzodiazepines in oral fluid (ng/mL).

Analytical evaluation for cannabinoids

Criteria

All subjects were classified as being positive or negative for cannabis according to the following criteria:

Oral fluid: positive if THC was identified and quantified at or above 2 ng/mL neat saliva.

Blood: positive if THC was identified and quantified at or above 1 ng/mL blood.

Results

The performance of the different oral fluid testing devices compared with the results of the confirmation in oral fluid is given in Table 8. Overall, the onsite assays detected only 46% of the THC-containing oral fluid samples.

Table 8: Oral fluid devices versus confirmation in oral fluid for cannabis.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	1659	722	170	22	44	29	44	37	591
Prevalence	36.0%	31.2%	13.5%	18.2%	61.4%	17.2%	45.5%	48.6%	46.5%
TP	276	76	17	1	8	0	13	6	155
TN	974	457	146	18	16	22	14	17	284
FP	84	40	1	0	1	2	6	2	32
FN	321	149	6	3	19	5	7	12	120
Sensitivity	46.2%	33.8%	73.9%	25.0%	29.6%	0.0%	65.0%	33.3%	56.4%
Specificity	92.1%	92.0%	99.3%	100.0%	94.1%	91.7%	70.0%	89.5%	89.9%
Accuracy	75.5%	73.8%	95.9%	86.4%	54.5%	75.9%	67.5%	62.2%	74.3%
PPV	76.7%	65.5%	94.4%	100.0%	88.9%	0.0%	68.4%	75.0%	82.9%
NPV	75.2%	75.4%	96.1%	85.7%	45.7%	81.5%	66.7%	58.6%	70.3%

The performance of the different oral fluid testing devices compared with the results of the confirmation in blood is given in Table 9.

Table 9: Oral fluid devices versus confirmation in blood for cannabis.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	952	530	8	4	35	19	35	35	286
Prevalence	42.0%	30.9%	50.0%	0.0%	42.9%	42.1%	45.7%	40.0%	62.6%
TP	201	75	2	0	2	0	12	4	106
TN	488	326	4	4	17	10	14	18	95
FP	63	40	0	0	3	1	5	3	11
FN	199	89	2	0	13	8	4	10	73
Sensitivity	50.3%	45.7%	50.0%	/	13.3%	0.0%	75.0%	28.6%	59.2%
Specificity	88.6%	89.1%	100.0%	100.0%	85.0%	90.9%	73.7%	85.7%	89.6%
Accuracy	72.5%	75.7%	75.0%	100.0%	54.3%	52.6%	74.3%	62.9%	70.5%
PPV	76.1%	65.2%	100.0%	/	40.0%	0.0%	70.6%	57.1%	90.6%
NPV	71.0%	78.6%	66.7%	100.0%	56.7%	55.6%	77.8%	64.3%	56.5%

The Box and Whisker Plot for the cannabis results of all devices compared to the concentration of THC in oral fluid is given in Figure 6. All concentrations higher than 500 ng/mL were equated to 500 ng/mL.

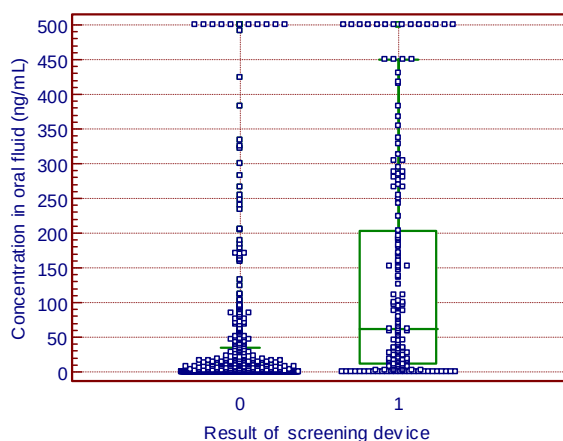
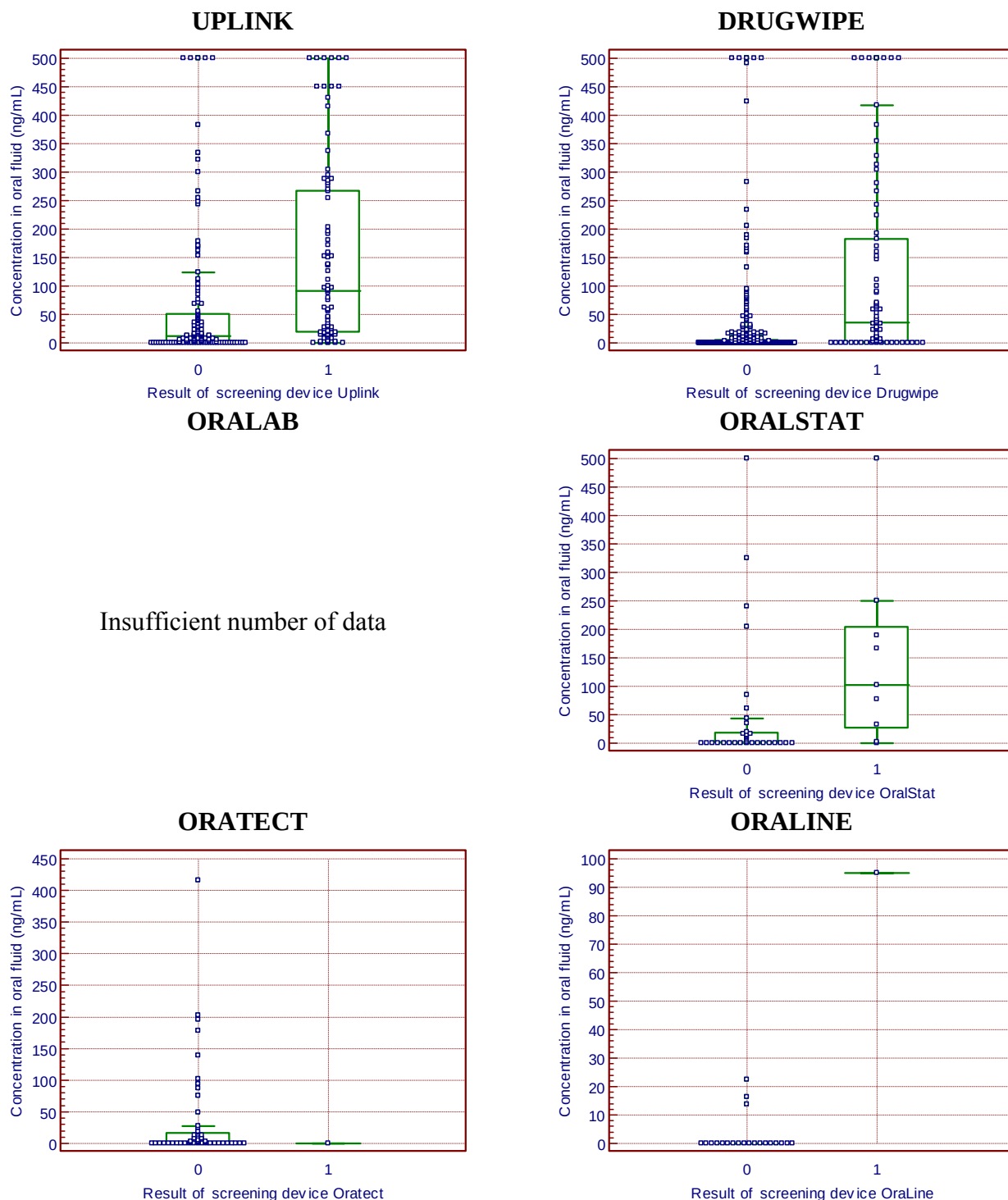


Figure 6: Box and Whisker Plot for the cannabis results of all devices compared to the concentration (ng/mL) of THC in oral fluid (0: onsite test negative; 1: onsite test positive). The values > 500 ng/mL were equated to 500 ng/mL.

The Box and Whisker Plot for the devices separately is given in Figure 7. All concentrations greater than 500 ng/mL were equated to 500 ng/mL.



Insufficient number of data

Figure 7: Distribution of the concentrations of the THC (ng/mL) according to the result of the onsite test (0: negative, 1: positive) for the individual devices. In order to get a better picture of the data, the values > 500 ng/mL were equated to 500 ng/mL.

The cumulative distribution of the concentration (> 0ng/mL) of THC in oral fluid is given in Figure 8. This figure illustrates that the values of THC in oral fluid are low. Fifty % of the THC values are lower than 35 ng/mL.

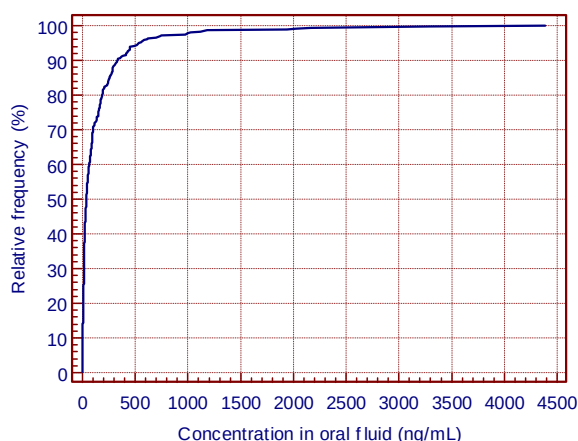


Figure 8: Cumulative distribution of the concentration (> 0ng/mL) of THC in oral fluid (ng/mL).

Analytical evaluation for cocaine

Criteria

All subjects were classified as being positive or negative for cocaine according to the following criteria:

Oral fluid: positive if cocaine or its metabolite benzoylecgonine (BE) were identified and quantified at or above 8 ng/mL neat saliva.

Blood: positive if cocaine or BE were identified and quantified at or above 20 ng/mL blood.

Results

The performance of the different oral fluid testing devices compared with the results of the confirmation in oral fluid is given in Table 10.

Table 10: Oral fluid devices versus confirmation in oral fluid for cocaine and benzoylecgonine.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	1669	725	187	24	44	29	40	37	583
Prevalence	19.1%	15.4%	19.3%	4.2%	9.1%	24.1%	22.5%	32.4%	23.5%
TP	246	77	35	0	2	3	6	9	114
TN	1306	590	146	19	38	22	29	25	437
FP	79	23	5	2	2	0	2	0	45
FN	72	35	1	1	2	4	3	3	23
Sensitivity	77.4%	68.8%	97.2%	0.0%	50.0%	42.9%	66.7%	75.0%	83.2%
Specificity	94.3%	96.2%	96.7%	90.5%	95.0%	100.0%	93.5%	100.0%	90.7%
Accuracy	91.1%	92.0%	96.8%	86.4%	90.9%	86.2%	87.5%	91.9%	89.0%
PPV	75.7%	77.0%	87.5%	0.0%	50.0%	100.0%	75.0%	100.0%	71.7%
NPV	94.8%	94.4%	99.3%	95.0%	95.0%	84.6%	90.6%	89.3%	95.0%

The performance of the different oral fluid testing devices compared with the results of the confirmation in blood is given in Table 11.

Table 11: Oral fluid devices versus confirmation in blood for cocaine and benzoylecgonine.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	725	432	8	6	35	19	35	35	155
Prevalence	15.7%	13.4%	12.5%	0.0%	11.4%	10.5%	11.4%	22.9%	23.9%
TP	87	43	1	0	2	2	4	8	27
TN	573	351	6	4	29	17	28	27	111
FP	36	23	1	0	2	0	3	0	7
FN	27	15	0	0	2	0	0	0	10
Sensitivity	76.3%	74.1%	100.0%	/	50.0%	100.0%	100.0%	100.0%	73.0%
Specificity	94.1%	93.9%	85.7%	100.0%	93.5%	100.0%	90.3%	100.0%	94.1%
Accuracy	91.3%	91.2%	87.5%	100.0%	88.6%	100.0%	91.4%	100.0%	89.0%
PPV	70.7%	65.2%	50.0%	/	50.0%	100.0%	57.1%	100.0%	79.4%
NPV	95.5%	95.9%	100.0%	100.0%	93.5%	100.0%	100.0%	100.0%	91.7%

The Box and Whisker Plot for the cocaine results of all devices compared to the sum of the concentration of cocaine and BE in oral fluid is given in Figure 9. All sums of concentrations higher than 5000 ng/mL were equated to 5000 ng/mL.

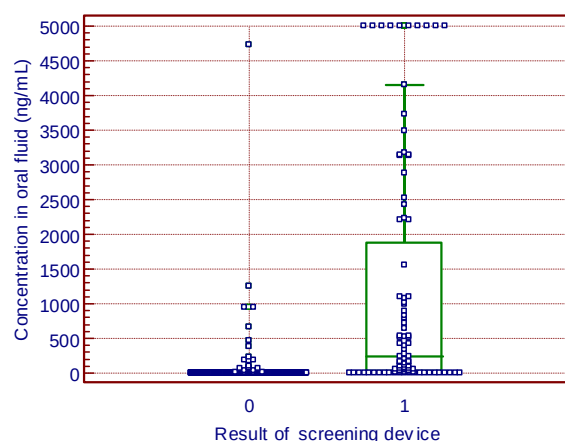


Figure 9: Box and Whisker Plot for the sum of the concentration of cocaine and BE in oral fluid (ng/mL) compared to the result of the on-site tests (0: negative; 1: positive). All concentrations > 5000 ng/mL were equated to 5000 ng/mL.

The Box and Whisker Plot for the devices separately is given in Figure 10.

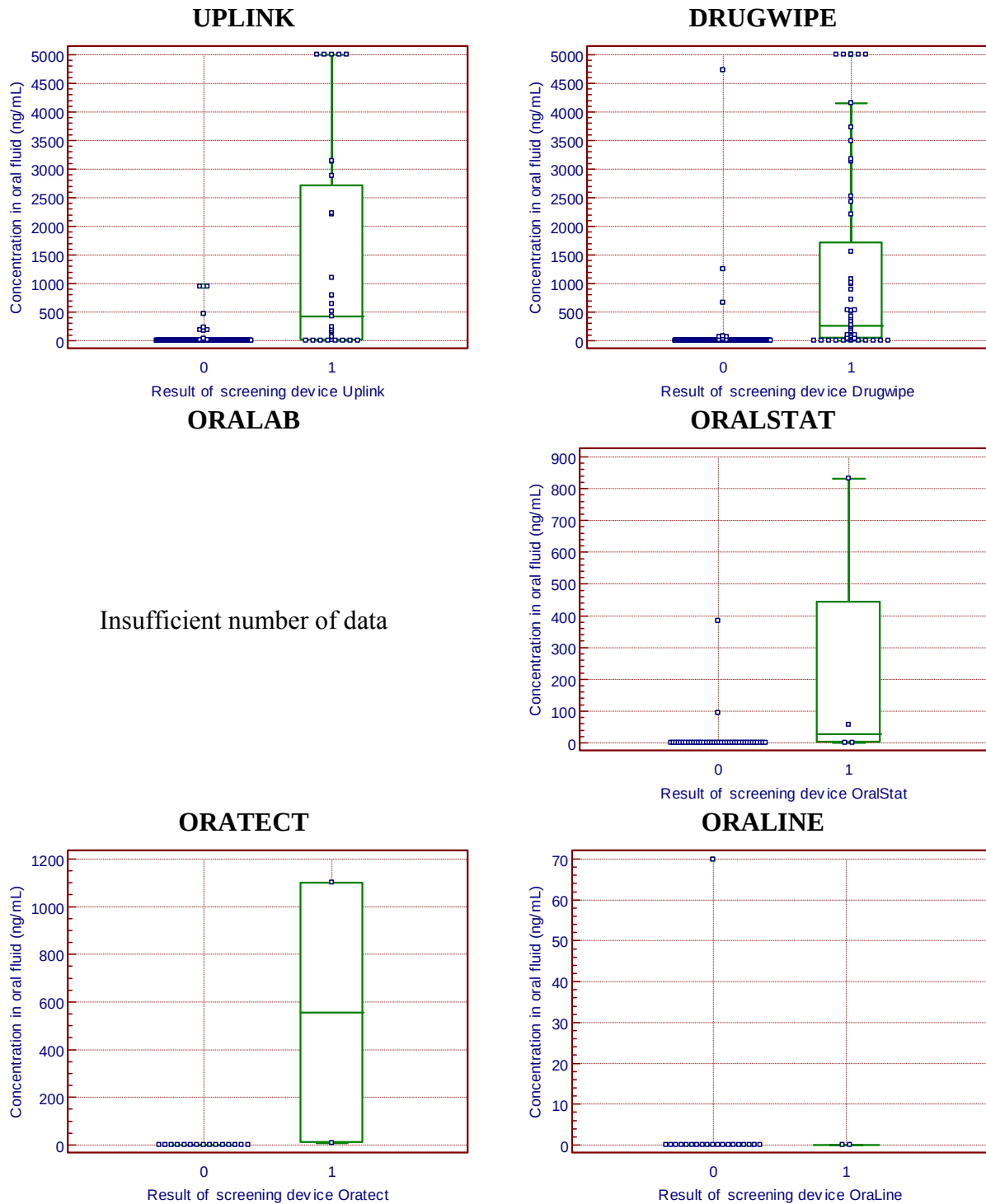


Figure 10: Distribution of the sum of the concentrations of cocaine and BE (ng/mL) according to the result of the onsite test (0: negative, 1: positive) for the individual devices. All sums of concentrations > 5000 ng/mL were equated to 5000 ng/mL.

The cumulative distribution of the sum (>0 ng/mL) of the concentrations of cocaine and BE is given in Figure 11. This shows that the concentrations of cocaine and BE are rather high. Fifty % of the values are higher than 447 ng/mL.

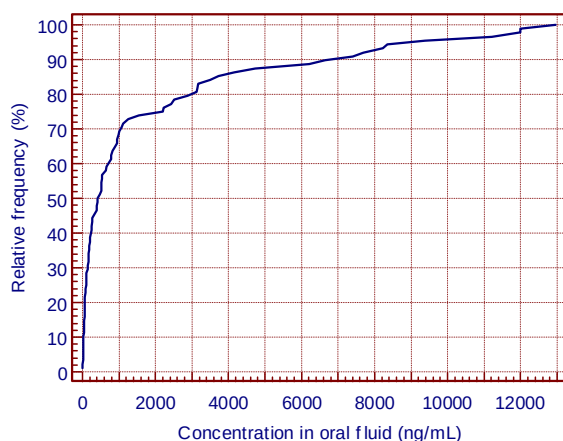


Figure 11: Cumulative distribution of the sum (> 0 ng/mL) of the concentrations of cocaine and BE in oral fluid (ng/mL).

Analytical evaluation for opiates

Criteria

All subjects were classified as being positive or negative for opiates according to the following criteria:

Oral fluid: positive if morphine or codeine were identified and quantified at or above 20 ng/mL neat saliva.

Blood: positive if morphine or codeine were identified and quantified at or above 10 ng/mL blood.

Results

The performance of the different oral fluid testing devices compared with the results of the confirmation in oral fluid is given in Table 12.

Table 12: Oral fluid devices versus confirmation in oral fluid for opiates.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	1667	725	187	22	44	29	40	37	583
Prevalence	7.7%	9.5%	3.7%	68.2%	0.0%	20.7%	15.0%	16.2%	3.3%
TP	78	35	7	13	0	5	4	4	10
TN	1516	640	180	6	42	23	34	30	561
FP	20	16	0	1	2	0	0	1	0
FN	50	34	0	2	0	1	2	2	9
Sensitivity	60.9%	50.7%	100.0%	86.7%	/	83.3%	66.7%	66.7%	52.6%
Specificity	98.7%	97.6%	100.0%	85.7%	95.5%	100.0%	100.0%	96.8%	100.0%
Accuracy	95.8%	93.1%	100.0%	86.4%	95.5%	96.6%	95.0%	91.9%	98.4%
PPV	79.6%	68.6%	100.0%	92.9%	0.0%	100.0%	100.0%	80.0%	100.0%
NPV	96.8%	95.0%	100.0%	75.0%	100.0%	95.8%	94.4%	93.8%	98.4%

The performance of the different oral fluid testing devices compared with the results of the confirmation in blood is given in Table 13.

Table 13: Oral fluid devices versus confirmation in blood for opiates.

	Total	Drugwipe	OraLab	OraLine	OralStat	Oratect	RapiScan	SalivaScreen	Uplink
Total	679	411	8	4	35	19	35	35	132
Prevalence	7.5%	5.4%	12.5%	50.0%	0.0%	26.3%	14.3%	5.7%	10.6%
TP	42	19	1	2	0	3	4	2	11
TN	601	372	6	1	34	12	30	30	116
FP	27	17	1	1	1	2	0	3	2
FN	9	3	0	0	0	2	1	0	3
Sensitivity	82.4%	86.4%	100.0%	100.0%	/	60.0%	80.0%	100.0%	78.6%
Specificity	95.7%	95.6%	85.7%	50.0%	97.1%	85.7%	100.0%	90.9%	98.3%
Accuracy	94.7%	95.1%	87.5%	75.0%	97.1%	78.9%	97.1%	91.4%	96.2%
PPV	60.9%	52.8%	50.0%	66.7%	0.0%	60.0%	100.0%	40.0%	84.6%
NPV	98.5%	99.2%	100.0%	100.0%	100.0%	85.7%	96.8%	100.0%	97.5%

The Box and Whisker Plot for the opiate results of all devices compared to the sum of the concentrations of morphine and codeine in oral fluid is given in Figure 12. All sums of concentration higher than 1600 ng/mL were equated to 1600 ng/mL.

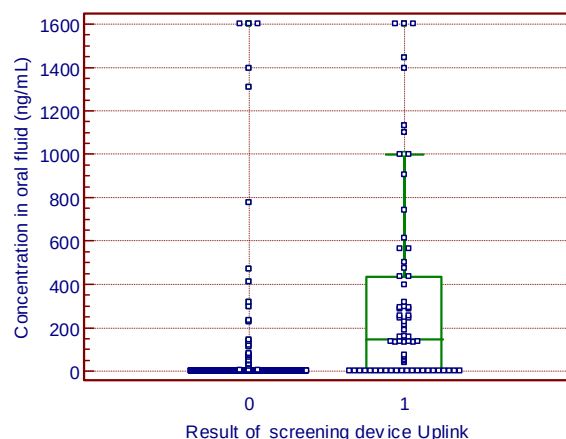
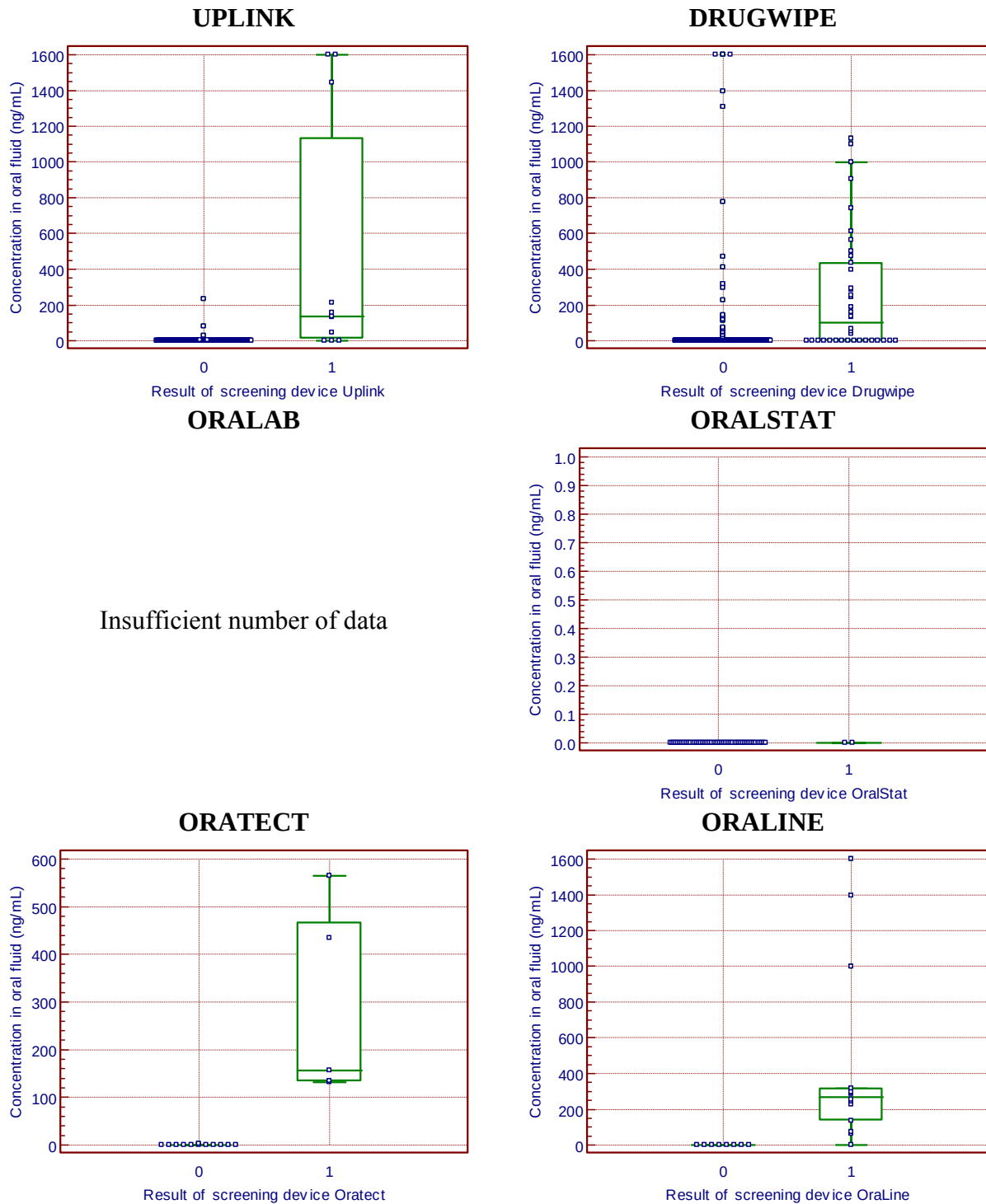


Figure12: Box and Whisker Plot for the sum of the concentrations of morphine and codeine in oral fluid compared to the result of all on-site tests (0: negative; 1: positive). All sums of concentrations > 1600 ng/mL were equated to 1600 ng/mL.

The Box and Whisker Plot for the devices separately is given in Figure 13.



Insufficient number of data

Figure 13: Distribution of the sum of the concentrations of morphine and codeine (ng/mL) according to the result of the onsite test (0: negative, 1: positive) for the individual devices. All sums of concentrations > 1600 ng/mL were equated to 1600 ng/mL.

The cumulative distribution of the sum (>0ng/mL) of the concentrations of morphine and codeine in oral fluid is given in Figure 14. This figure illustrates that the opiate concentrations in oral fluid are relatively high. Fifty % of the values are higher than 247 ng/mL.

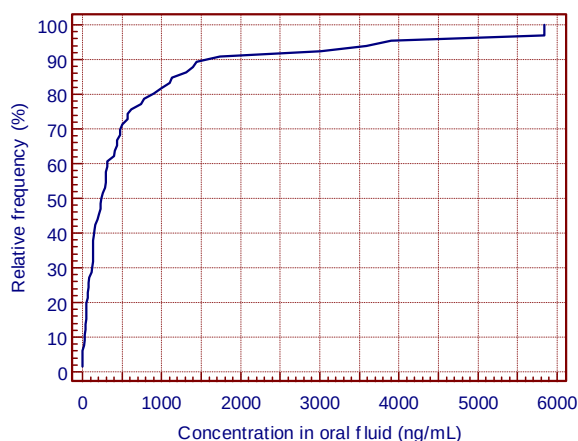


Figure 14: Cumulative distribution of the sum (>0ng/mL) of the concentrations of morphine and codeine in oral fluid (ng/mL).

Comparison of the results of the lab analyses on oral fluid and blood in order to estimate the best cut-off

Methods

We also determined what would be the ‘optimal’ cut-off for the confirmation of the analytes in oral fluid, in order to ‘predict’ that the blood analysis would be positive for these analytes. Our reference method is thus the lab analysis of the analytes in blood.

For the calculation of the receiving operating characteristic (ROC)-curves, the concentrations of the following analytes in oral fluid and blood were used:

- Amphetamines: amphetamine, methamphetamine, MDMA, MDA and MDEA
- Benzodiazepines: diazepam, nordiazepam, oxazepam, temazepam, alprazolam, lorazepam and clonazepam
- Cannabis: Δ^9 -THC
- Cocaine: cocaine and benzoylecgonine
- Opiates: morphine and codeine

Results

The number of paired samples, the optimal cut-off, the sensitivity and specificity at that cut-off and the area under the ROC-curve (AUC) are given in Table 14. For amphetamines, cannabis, cocaine and opiates the AUC under the ROC-curve is > 0.92, which illustrates that oral fluid is a good screening fluid for the presence or absence of these drugs in blood. For benzodiazepines, there is not a good relationship between the presence of drugs in oral fluid and in blood.

The table also shows that the cut-offs concentrations are low, less than 10 ng/mL for all analytes except amphetamines.

Table 14: Determination of the optimal cut-off for the (sum of) the analytes in oral fluid in order to detect the subjects in whose blood the drugs can be found above the cut-offs given in Table 3.

Analyte	n	Optimal cut-off (ng/mL)	Sensitivity at that cut-off (%)	Specificity at that cut-off (%)	Area under the ROC-curve
Amphetamines	325	160.40	94.7	97.8	0.979
Benzodiazepines	183	3.00	63.8	93.2	0.787
Cannabis	518	3.60	88.9	82.0	0.924
Cocaine	324	8.20	95.3	96.8	0.986
Opiates	309	3.16	95.0	96.9	0.965

Operational aspects

An important part of Rosita-2 was the evaluation of the practical aspects of the use of the different oral fluid devices by the police officers.

The next paragraphs give an overview of the comments that were made by the police officers in the different countries and states.

Securetec Drugwipe

Police officers in Finland have been satisfied with the practical aspects (size, practicability, rapidity and portability of testing device) of Drugwipe, because it fulfils at least partially these requirements. What was also very practical was that devices could be delivered to different policemen and they could put them into their pockets.

In several other countries, the feedback from officers that used this device was also positive. They reported the device was **easy to handle**, the oral fluid was gathered quickly and it was not very intrusive. In Utah the Drugwipe device was considered acceptable for roadside testing based on its ease of use and the willingness of the donors to provide specimens.

The use of Drugwipe gave some **read-out problems**, resulting in a difficult interpretation (see figure 15). Also in Finland the results from the cannabis test were generally considered to be quite difficult to interpret. In Finland, the thin red colour of cannabis lines (indicating a positive test) was considered to carry a risk of false positives. This was confirmed in Norway: an indistinct colour change line made the reading very difficult, particularly outdoors with poor lighting. An increased contrast with more distinct colour line (blue or black) was considered to be necessary. Using an intense flashlight may be helpful for the interpretation of the results, but it did not significantly improve the readability.

Practical problems have been noted in Finland and Norway with, e.g., using cold water for testing and/or test devices at winter time. The water bottle makes the devices less user-friendly and not practical during cold wintertime. Contaminations of the water may occur and therefore influence on the results. An incorporated sealed container in the package with sufficient fluid that also works below zero degree temperature would be preferable.

In Norway, police estimated that the devices could not be recommended for a **temperature below zero** as time from sampling to available results increased significantly (10 – 15 min or more), showing several invalid results. Freezing weather was also a concern in Washington. Testing during **heavy raining** made the reading even more complicated with raindrops in the reaction area. A transparent protective cover would solve this problem.

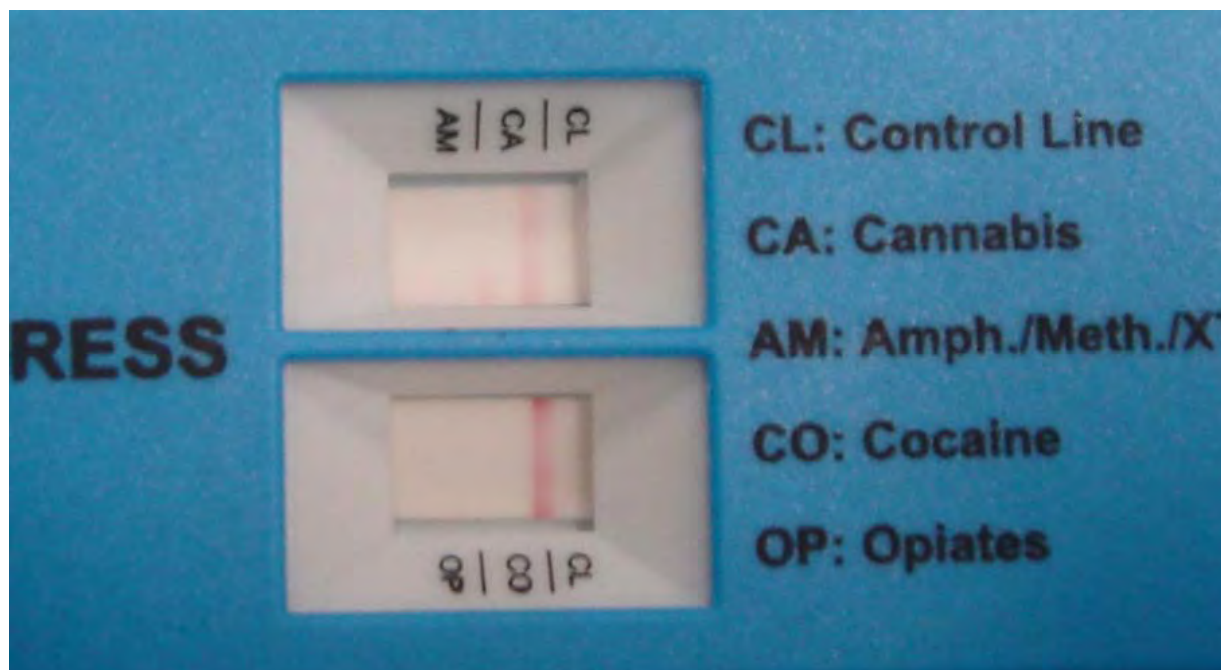


Figure 15: picture a Drugwipe used in Belgium in November 2005. The upper result window shows a very faint line (a little bit to the left) of the CA (for cannabis) mark. This was considered a positive result.

The testing time (evaluated to be 5-15 min) was considered acceptable by most of the officers in Finland when dealing with a person suspected of driving under the influence of drugs. This was also the case in Belgium. Many police officers in Finland have reported that the detection of cannabis takes generally much longer than the other drugs (10-15 min).

In Finland, the more experienced police officers were more satisfied with the Drugwipe, and they appreciated the ease of use, credibility of the test, interpretation of the results and the usability of the test for on-site testing. Furthermore, there were less test failures among the more experienced group. All police officers were willing to continue to use Drugwipe. In Norway, the testing procedure was considered to be easy because of the few steps. The inclusion of benzodiazepines in the test menu was also appreciated, but police officers would prefer to have all drug groups on one device.

A small and user-friendly reader could be desirable but it was not the primary demand in Norway.

Police officers also had concerns about the high number of false negatives for cannabis, cocaine and benzodiazepines.

Dräger DrugTest/Orasure Uplink

This device was considered too time-consuming (15-20 minutes) in Belgium.

The **sample collection** with the collection sticks of the Dräger DrugTest® was hygienic and easy, especially because the status of the procedure could easily be checked by looking at the expansion grade of the sponge: if not sufficient, the collection could be continued.

None of the **invalid tests** was due to difficulties in sample collection. The reading of the results with Dräger DrugTest® was regularly not possible because no reading unit was available or the reading unit had a software problem and could not read the test cassette. The software was updated so that problem should have been limited.

The **handling** of the DrugTest was **easy** but an instrument was necessary to read the results. That meant that this test was not completely portable. This was confirmed in the US: the

instrumented “UpLink” device worked well in a stable setting such as the Batmobile or the centralized BAT center where sufficient room and power were available.

In Germany the Dräger DrugTest was very well accepted by police officers and tested persons, even though a reading unit was necessary.

The reader could only be used on the police station and was not suitable for roadside use in Norway.

In e.g. Spain, police officers did appreciate the **automatic interpretation** and printed result, which was preferred to a manual interpretation. The police officials in Spain very categorically rejected a subjective interpretation of the test.

In a number of countries, the Dräger Drugtest was considered to be rather **complicated** to use with many steps before the results were available after at least 16 minutes. Even with good instruction, it was easy to make a mistake that required restarting the process, adding 8-12 minutes before the final results. It was necessary to get sufficient time for training; several tests were needed before they were familiar with the system. Police officers did appreciate the good support given by the manufacturer.

The duration of the procedure (at least 15 minutes before the results are available) was considered too long in Norway.

According to the Norwegian police officers, the benzodiazepines should be added to the menu.

The device was not acceptable for pre-screening of suspected drugged drivers in Norway. For this device as well, there was a concern about the insufficient sensitivity for cannabis.

Branan Oratect

The first version of the Oratect had great difficulties in sample collection. The amount of oral fluid collected for a valid test was mostly not enough and the procedure too complicated so in most cases no testing was possible. The sample collection time was much longer than 5 minutes. It seemed that the test persons could outsmart the test by not following the instructions. A high number of invalid results were also observed in Norway. During training of the police, it was difficult to get sufficient oral fluid on the collection pad, even after keeping the device in the mouth for much longer time than recommended by the instructions. In some cases, oral fluid was absorbed on only half of the collection pad. A new batch of the device did not solve the problem.

Branan Medical provided an improved version of the test after a few months and the new test had a shorter collection pad, so less oral fluid was necessary. The number of tests that collected enough oral fluid was much higher but still not sufficient.

If the tests were working the results could easily be read without any difficulties.

The time before reading of the results should be shorter and (in Norway) benzodiazepines should be added.

The devices could not be used because of too many invalid results.

There were also concerns that the device was not sensitive and accurate enough to detect the two primary drugs of abuse, i.e. marijuana and cocaine.

According to the German police, the Oratect would be an ideal test system, because it is very small and portable; every police officer could take some tests with him during his routine work. No additional equipment is needed. But the sample collection is too complicated, it can be outsmarted by the tested persons and it takes too much time.

Sun Biomedical Oraline

The test procedure for Oraline[®] was simple with few steps. A drawback was that a rather large sample volume was needed which may be difficult for drug abusers. It seemed that the time for the sample to move from collection area was dependent on the temperature. Cold weather (approximately 0 °C) increased the time for the sample to move from collection pad to the reaction area with antibodies. The lines indicating positive or negative results were often too pale for clear conclusion. Some tests were performed during heavy raining, and raindrops fell into the reaction area. It was then impossible to read the results. A transparent protective cover would solve this problem. The time from sampling to final results was too long to be used for roadside control. In Norway benzodiazepines should be added. The boxes for storing the devices were unnecessary. The testing instructions could be printed on the plastic cover instead of the boxes.

Varian Oralab

The ORALAB device was simpler to handle than e.g. the Dräger Drugtest. In Wisconsin, subjects were often unable to provide sufficient oral fluid during specimen collection, resulting in many invalid tests. The high rate of invalid tests influenced some officers to lose enthusiasm for the study and stop recruiting subjects. Officers also experienced difficulty observing the presence or absence of the test lines making interpretation of results inconsistent.

Lifepoint[®] IMPACT[®]

The Lifepoint[®] IMPACT[®] used a new way of sample collection, comparable to a saliva aspiration pump at the dentist's. This seemed to provide an exact amount of oral fluid for the testing procedure. However, in many cases the collected sample volume was not sufficient because the instrument stopped the sampling automatically after a preset time, which could not be extended, even if not enough oral fluid was collected.

American Biomedica OralStat

The collection stick of the OralStat[®] lost one of its collection sponges in some cases. This test could also be read without the reading unit, although one was available. The scanning of the test strip by the electronic reader was sometimes difficult because the system showed wrong results if the test strip was not in the exact position. This made the handling difficult for the officers.

Cozart RapiScan

The operating procedure of the RapiScan is fairly direct, but was found to intimidate officers if they were not able to use it soon after training. Many officers indicated they were uncomfortable using the instrument, stating that it was difficult to remember the procedure and that they would have preferred to use a non-instrumented or manually interpreted device.

Ultimed SalivaScreen

The SalivaScreen 5TM device gave more invalid tests than it did valid. Officers reported the result bands smearing or not enough saliva collected by the device to give a reading. Feedback from officers also indicated that the device was not practical and needed to be simplified. Specifically, that it took two minutes to collect the sample, having to manually

transfer saliva from collection device to reading device, and then waiting an additional ten minutes for the device to produce results.

The test kits were required to be kept at room temperature. In many areas, during the winter months the temperature is below the recommended storage temperature of 2°C. This prevents those officers from carrying the devices in their car.

General comments about the use of oral fluid for roadside drug detection.

In Belgium, police officers felt that use of oral fluid would facilitate the legal procedure. The principle of the Drugwipe® (wiping the tongue) was highly appreciated. Complex sampling devices such as the Dräger DrugTest® were less well accepted.

In Germany, it was concluded that at present none of the evaluated test systems for oral fluid is acceptable for the police. However, the collection of the oral fluid samples with Dräger, Branam, Lifepoint and ABMC was acceptable for the police officers: the handling was hygienic and no direct contact with the sample was necessary.

For the Norwegian police, improved user-friendliness and increased sensitivity for some important drugs are necessary, before the authorities can accept the equipment to be used by the police.

In Spain, a device that does not require a subjective interpretation is preferred. The duration of the test is not a critical condition, but the precision and reliability are highly prized. Failures in the device reader or function are one of the more important disadvantages according to the police.

Impact on National Legislation

An unexpected aspect of the Rosita-2 study was that three countries stated that the study had an impact on the legislation on driving under the influence of drugs (DUID).

Finland:

One of the most important outcomes of the Rosita-2 project in Finland was that several proposals for new orders, regulations and instructions were made. A regulation from the Ministry of Interior enacted that on-site oral fluid screening tests for drugs were considered equal to on-site breath alcohol tests, and were thus compulsory, if asked by a police officer. Other orders and regulations from the Ministry of Interior were modified, such as “*Supervision of drug use in road traffic*”, “*Drug dependence and driving permission*” and new “*field sobriety observation sheet*” for police officers. All these changes were mainly results of the ROSITA projects.

Norway:

The Norwegian authorities represented by the Ministries of Police, Transport and Health want to prioritize equipment for roadside screening as a part of an increased action against drugged driving and to simplify the police work. New devices that satisfy the requirements from the police including good correlation with blood samples are welcome. Onsite devices are especially mentioned in the proposal from a working group appointed by the Ministry of Transport. The aim is equipment with similar user-friendliness and time use as an alcoholmeter for testing of suspected drivers.

Spain

The participation of Spain in the ROSITA I and II projects had positive consequences. Similarly to other European countries, the responsible authorities of Road Traffic in Spain are preparing legislation on DUID. It is the first time that the General Directorate is trying to change the law in order to allow in some cases to take a blood sample, in order to document driving under influence of a drug by the presence of this drug in the blood of the driver. An onsite device is important to provide the police officers with a presumptive evidence of recent drug use.

Conclusion

The Rosita-2 study was a three-year, international (6 countries in Europe and 4 American states) evaluation of onsite oral fluid devices.

Nine on-site oral fluid devices were evaluated. For six of them, the number of failures was greater than 25%, which is unacceptably high.

The analytical evaluation (in comparison to reference methods in oral fluid and/or blood) showed that for the amphetamine and methamphetamine tests the sensitivity varied between 40% and 83% and the specificity between 80% and 100%. For the benzodiazepine tests the sensitivity was 33-69% and the specificity 85-94%. For the cannabis tests the sensitivity was 0-74% and the specificity 70-100%. Detailed analysis of the data for cannabis showed that some devices (e.g. Drugwipe) gave a negative result even when very high concentrations of THC were found with the Intercept. For the cocaine tests, the sensitivity was 0 - 97% and the specificity 91 - 100%. Finally, for the opiates, the sensitivity was 51 - 100% and the specificity 86 - 100%. No device met the criteria proposed during the Rosita-1 project (sensitivity and specificity > 90%, accuracy > 95%) for the amphetamines, benzodiazepines and cannabis. The Varian Oralab met these criteria for cocaine and opiates, but it gave 26% failures, so it cannot be recommended.

Analysis of the distribution of the drug concentrations in oral fluid showed that 50% of the positive samples were below 2598 ng/mL for the sum of the amphetamines, 25 ng/mL for the sum of the benzodiazepines, 35 ng/mL for THC, 447 ng/mL for the sum of cocaine and benzoylecgonine and 247 ng/mL for the sum of codeine and morphine.

A comparison of the results of the lab analyses on oral fluid and blood in order to estimate the best cut-off showed that oral fluid is a good screening fluid ($AUC > 0.92$) for the presence or absence of amphetamines, cannabis, cocaine and opiates in blood. For benzodiazepines the AUC is lower.

The evaluation of the operational aspect showed that sampling with some devices was easy and hygienic, but it was more difficult with others. Use of some devices in cold and rainy weather is a problem. In general police officers preferred an electronic reader, but it is an advantage if the device can also be read without the device.

The testing procedure for some devices was considered too lengthy and complicated.

At the end of the study, no device was considered to be reliable enough in order to be recommended for roadside screening of drivers. However, the experience in the state of Victoria in Australia shows that random roadside oral fluid testing of drivers for methamphetamine and cannabis (using the Securetec Drugwipe followed by the Cozart RapiScan and chromatographic analysis in the lab) has a deterrent effect. Government officials should carefully weigh the pros (deterrent effect) and the cons (risk that drivers will realise that they often test negative after having used drugs due to the limited sensitivity of the test) of introducing random drug testing with the currently available devices.